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OSMOSIS EXPERIMENTS WITH LIVING AND DEAD MEMBRANES.

By E. WAYMOUTH REID.

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OSMOSIS EXPERIMENTS WITH LIVING AND DEAD MEMBRANES.

By E. WAYMOUTH REID, *Professor of Physiology at University College, Dundee, University of St Andrews. Late Assistant Lecturer on Physiology to St Mary's Hospital Medical School.* PLATES VIII., IX., X., XI.

MANY observers have borne witness to the fact, that the phenomena of Diffusion between two liquids separated by an animal membrane, present differences, according as the membrane has been recently removed from the living body, or has had time to undergo various post mortem changes prior to its employment for experiment.

Dutrochet[1], in some of his earlier experiments with the cæcum of the fowl, noticed that the freshness of the viscus influenced the result of the experiment. Thus, he found that the fresh cæcum filled with milk and plunged into water, continued to increase in weight for a period of 36 hrs.; in a second experiment with the same cæcum and fresh milk, the increase of weight continued only for 24 hrs., and the amount of fluid introduced into the organ by osmosis was only about one fifth part of the amount passing when the specimen was perfectly fresh.

Matteucci and Cima[2] experimenting with the skins of the frog, the eel, and the torpedo, found that variations in the facing of the surfaces of the skin towards the fluids used for experiment, exerted an influence upon the rapidity of osmotic transference of fluid, and that this peculiarity existed only while the membranes were in a fresh state. They also observed similar differences in the vesical mucous membrane of the ox, and the gastric mucous membrane of the lamb, the cat, and the dog.

Claude Bernard[3] showed that the excised mucosa of the stomach is capable of offering a resistance to the passage of curare, while it is in a living condition; removal of the epithelium robbed it of this property.

[1] *L'Agent immédiat du Mouvement Vital*, Paris, 1826.

[2] *Ann. de Chimie et de Physique*, t. XIII. 1845.

[3] *Union Médicale*, pp. 445, 457, 462, 1849.

(Von Wittich[1] mentions that curare was given by the mouth as an antipyretic by Richard Schomburgk in British Guiana in the years 1840 to 1844.)

I have obtained a record (Tracing 1, Pl. IX.) of this fact in the following manner. To the lower ends of two tubes having a sectional area of 95 sq. mm. and a capacity of 10 c.c. were tied the gastric mucous membranes of two frogs; in one case the membrane was perfectly fresh, while in the other it had remained 16 hrs. in normal saline solution before employment for experiment. The tubes were fixed vertically in a moist chamber and were filled with normal saline. The two gastrocnemii nerve-muscle preparations of a frog were suspended by their femora, so that one muscle was immersed in the salt solution in each tube, while the sciatics passed over a pair of electrodes so arranged that the stimulus was of the same intensity to each of the two nerves. A pair of rigid wires attached to the tendons of the muscles passed vertically upwards and through the roof of the moist chamber; these wires actuated two levers whose movements were recorded on a travelling smoked surface. The lower ends of the tubes covered by the mucosæ were dipped into a vessel containing ·5% solution of curare in normal saline, and the response of the muscles to stimulation of their nerves was tested from time to time, the secondary coil of the induction machine being at 20 cm. from the primary and the battery being one Daniell cell. Three hours after the commencement of the experiment the muscle in the tube covered by the stale mucous membrane showed signs of curarization, and by 3 hrs. and 30 minutes it refused to respond to the stimulus, at which period however the muscle in the tube closed by fresh mucosa contracted vigorously to the same stimulus. The strength of the induction shock was then gradually increased until the primary coil was completely enclosed by the secondary; such increase of stimulus however failed to call forth a contraction of the muscle in the tube closed by stale mucous membrane, while that in the tube closed by fresh mucous membrane still contracted and indeed showed no sign of curarization. I have repeated this experiment four times and have always obtained the same result.

Susini[2], using the bladder of the rabbit filled with a 5% solution of potassium ferrocyanide, found that in the fresh state the salt did not appear upon the outer surface until about one hour after the commence-

[1] *Handbuch der Physiologie*, Bd. v. Th. II., Hermann.

[2] *Journal de l'Anatomie*, p. 144, 1868.

ment of the experiment, but that erosion of the epithelium greatly hastened its passage through the wall of the organ. He also noted a much faster passage of the salt through the wall of the intestine than through that of the stomach.

Cazeneuve and Livon[1] experimented with the exsected bladders of dogs which had been compelled to retain their urine for some hours before they were killed. The bladders, distended with urine, were suspended in distilled water, and the hypobromite of soda test applied to a portion of the water from time to time in order to detect the presence of diffused urea. From a fresh bladder they found that no urea had passed out by 4 hrs. after the commencement of the experiment. After allowing 24 hrs. to elapse before using the bladder, the presence of urea could be detected in from 10 to 15 minutes. Scraping away epithelium from within by means of a sound at once rendered the wall permeable to the contained solution of urea. They noticed moreover that the state of nutrition of the animal influenced this retarding action of the epithelium of the bladder.

Englemann[2], in some experiments relating to the influence of the membrane upon the process of electro-osmosis, found that the fresh skin of the frog was less permeable than skin which had remained in water for some days prior to its use.

I have repeated the experiment of Cazeneuve and Livon with the bladder of the cat, suspending it in distilled water as soon as practicable after decapitation. With a normal bladder, I found no trace of urea in the surrounding water till 5 hrs. after death, by using the hypobromite of soda test; in another case the wall of the bladder was rubbed between the finger and thumb in order to detach the epithelium; in this case urea was found within 2 hrs. after death.

It is evident then that diffusion through animal membranes is greatly influenced by the physiological state of the tissues composing such membranes, a fact daily brought before the notice of the physician in cases of albuminuria of diverse origin, various pathological transudations etc.

Experimental evidence has been given moreover that some power of selection resides in the protoplasm of the epithelia covering certain of the cavities of the body.

Tappeiner[3] in a series of very careful experiments conducted for

[1] *Comptes Rendus*, t. 87, p. 435, 1878.

[2] *Arch néerlandaises*, p. 332, 1874.

[3] *Wien Sitzungsberichte*, Bd. 77, Abtheil. III. p. 281, 1878.

the most part on dogs, found that whilst in the duodenum and jejunum injected taurocholate and glychocholate of soda were not absorbed, in the ileum both salts rapidly disappeared after their introduction. The absence of absorption in the first instance could not be traced to any chemical changes undergone by the salts, to injury to the parts or to the shock of the operation. The amount of salts absorbed in the ileum towards the end of an experiment of 3 or 4 hrs. duration was found to be less than during the earlier stages. He concluded that differences of selective power of epithelia could alone account for the facts observed, and that the diminution in rate of absorption observed towards the end of an experiment was a result of fatigue of the epithelial function.

The experiments of Susini quoted above, with reference to the greater ease with which potassium ferrocyanide passes through the wall of the intestine than through the wall of the stomach, seem to point to the same fact.

The experiments in the present research have been directed towards a more careful study of the influence exerted by variations in the physiological condition of an animal membrane upon the process of diffusion through its tissues. The skin and the gastric mucous membrane of the frog have been employed as membranes, but in the present paper I shall confine myself to the observations made with the former tissue.

In the process of absorption in the living body, vascular and perhaps nervous factors are at work, whose value cannot be gauged with exactness, though they profoundly modify the course of events. It is only by exclusion of these factors, for the sake of simplifying the problem, that one can hope to arrive at any clear and definite conclusion with regard to the part played by epithelial action in the process of transference of fluid through a living membrane. To this end the persistent vitality of the excised tissues of cold-blooded animals lends itself.

If diffusion experiments are to be conducted with exsected tissues, whose vital condition must be prolonged as much as possible, it will be necessary to make use of fluids having the smallest deleterious action as regards the life of the tissue, consistent with sufficiently low osmotic equivalent to give a fairly rapid indication of the transference of fluid through the membrane. In searching for such fluids the period of persistence of the movements of the cilia of the ciliated epithelium of the frog, when immersed in the fluid and protected from pressure, was taken as an index. It was finally determined that a 5% solution of

glucose in normal saline solution was the most convenient for general use; in this fluid cilia from the œsophagus of the frog continue in an active condition for from four to five hours.

Apparatus and Methods.

Osmometers of the following patterns were employed, and in all cases were worked in pairs simultaneously to eliminate as far as possible errors due to variation of temperature from day to day.

Elevation of temperature causes an increase in the rapidity of diffusion when a piece of dead membrane is employed for experiment, and Eekhard[1] has given a formula for the coefficient when ox pericardium and sodium chloride are employed; apart from the complication introduced, it would not appear safe to apply this formula, obtained from experiments, on what must be considered dead membrane, on account of the strengths of the solutions of salt, to experiments in which the membrane is alive at the commencement of the process, and continually altering in nature as the experiment takes its course.

1. The classical form of osmometer of Dutrochet, in which the process of osmosis commences at a pressure, increasing as the experiment proceeds, was in some cases employed. The pairs were constructed in the following manner. A piece of glass tube was cut across and the two ends of the section ground flat and polished; a groove was then cut round the side of the tube about ·5 millimetre from the lower edge by means of an emery wheel; the other end of each piece of tube, after being drawn in a flame, was fused on to a piece of tubing about 20 centimetres long. By this method it is possible to get the apertures of the two osmometers of exactly the same area; if the edge of the tube is turned out in a flame, in order to get a rim to prevent the slipping off of the membrane, a satisfactory pair cannot be obtained. The same pair has been used in all the experiments published, the measurements being, area of aperture 95 sq. mm., diameter of observation tube 3·5 mm., capacity up to mark 5·5 c.c.; a rise of 1 mm. of the column of fluid indicated an introduction of 9 cubic mm. of fluid into the reservoir through the membrane.

In all experiments the same volume (5·5 c.c.) of fluid was introduced into the apparatus, and the same parts of the observation tubes used; the parts of the tubes which corresponded were determined by experi-

[1] *Beiträge*, Bd. II. p. 27, 1860.

ment, the error from variation of calibre being so small that it could be safely neglected. The fluids employed were allowed to attain the room temperature before introduction into the instruments.

The membrane was carefully supported by resting upon an ebonite plate pierced with holes about ·5 sq. mm. in area; the circle of the base of the instrument included 40 of these holes, so that about 20 sq. mm. of membrane were exposed through the holes, though doubtless diffusion currents passed also between the plate and the membrane. These ebonite plates were clamped together during the operation of drilling in order that the same number of holes per square centimetre might be present in each. To test such a pair of osmometers, equal volumes of a solution of glucose in water were introduced into the reservoirs, three layers of Roy's membrane or collodion film tied on by waxed thread, and a scale in millimetres attached to the observation tubes; the membrane covered ends were immersed to the same depth in vessels containing 300 c.c. of distilled water, and rested on the perforated ebonite supports. If more than one millimetre difference was noted in the levels of the tops of the columns of fluid at the expiration of 24 hrs. the pair was discarded. By the method of making detailed above, it was found fairly easy to get a good pair; by the ordinary method by which a rim is formed in glass blowing it is extremely difficult. The employment of so small an area of membrane necessarily renders the rise of fluid in the observation tube very slow; it is however only by using small and well-supported surfaces, that bagging of the membrane can be prevented in osmometers working at a pressure.

(2) The second form of instrument, which I have termed the Oil Discharging Osmometer, is shown in fig. (1), Plate VIII. Its advantage over the Dutrochet pattern is that the pressure can be made nil at the commencement of the experiment, and any pressure due to fluid introduced by osmosis is at once relieved. The part of the apparatus to which the membrane is tied is prepared in the manner already described, in order that equal areas may be obtained in the two instruments forming a pair; a stopper is added for convenience of filling the tubes.

The stout arm (*A*) of the instrument is filled with the denser of the two solutions employed for experiment; the thin arm (*B*) is filled with a non-drying oil, and the length of the thin arm is adjusted by means of a piece of rubber tubing, so that the lengths of the arms are inversely proportional to the densities of the fluids

they contain. The membrane-covered end of the stout arm rests on the surface of the less dense fluid contained in a convenient vessel (*D*). It will be evident from this arrangement that the membrane can be placed at zero pressure at the commencement of the experiment. Beneath the open end of the thin arm is placed a small carefully weighed vessel, the oil receiver (*C*). As the process of osmosis proceeds the oil is forced out and falls into the oil receiver, the excess of the terminal over the initial weight of which indicates the amount of oil forced out of the apparatus. If the relation of volume to weight in the specimen of oil employed be known, the volume of fluid introduced by osmosis, and which has replaced the oil, can be easily determined. The pairs were tested with Roy's membrane or collodion film as described above. The same pair have been used in all the published experiments; in this pair a difference of 4 milligrammes in a discharge of 24 hours with collodion film and 5% glucose in water was the maximum error in trials in which from three to four decigrammes of oil passed out of the apparatus. This small error I have neglected, and in testing the pair from time to time I have not found it to be exceeded. The capacity of the pair used in the published experiments was 8·5 c.c. up to mark to which they were filled.

For recording purposes two forms of apparatus were used. The first form of apparatus employed (fig. 2), Plate VIII. records on one sheet of paper the rise of fluid in the two tubes of a pair of osmometers which are really only modifications of the Dutrochet instrument, except that the pressure is nil at the commencement of the experiment. The tubes are fixed vertically above one another in front of a vertical slit cut in the front board of the camera (*A*). A sheet of sensitized paper (Eastman's bromide paper in a roll of 50 ft. long, by 10 in. wide) is caused to pass behind the slit by the action of the eight-day clock (*B*); the paper rolls off a vertical cylinder connected with the pulley (*X*), on to a second vertical cylinder connected with the coned wheel (*Y*). The paper is kept strained by the action of a check weight acting on the pulley (*X*), and behind the slit passes over a small vertical roller, not shown in the figure. Any rise in the fluid in the recording tubes shows itself on development of the photographic paper by means of a white line, produced by the interception of the light of a paraffine lamp or Argand gas burner by the meniscus at the surface of the fluid. I at first employed a dark fluid (10% solution of aniline oil in turpentine) on the surface of the fluid in the observation tube, this did not however give a very sharp line always,

and I have to thank Prof. Schäfer for pointing out to me that the meniscus was sufficient to intercept the light. The clock makes one revolution in 12 hours, and by means of the cord passing round its driving pulley and the coned wheel (*Y*) various rates of movement of the paper can be employed; the speed generally employed was a movement of 6 mm. of paper past the slit in one hour. A projection on the hour axis of the clock causes the lever (*C*) to be raised once in every hour for a period of time sufficient to allow of a full exposure of the paper and so both mark the time and form a base line for measurement of the curves obtained. The membrane-covered ends of the osmometers either dip into a measured amount (300 c.c.) of the less dense fluid, or are fixed into vessels of the form shown at (*D*) in the figure, being fitted in with a caoutchouc cork; these vessels allow of a continuous change of the less dense fluid when connected by their inlet tubes with some vessel containing a supply, or they may be used to observe the effect of a gas upon the membrane, the fluid in them being saturated and the space between its surface and the cork being filled with the same gas, one of the tubes passing into the vessel being connected with a large vessel containing a supply. The membranes were supported on ebonite supports when the ordinary vessels were used; when the vessel shown at (*D*) was employed, a piece of Roy's membrane, which when used alone gives very constant results, was tied on over the experimental membrane. The capacity of the osmometers used in this apparatus was 8·275 c.c., the area of the lower aperture 95 sq. mm., diameter of recording tubes 3·5 mm.; a rise of 1 mm. indicated an introduction of 9 cubic mm. of fluid into the osmometer. (Tracings 6 to 12, Plate X., are reproduced $\frac{1}{3}$ natural size; thus in these $\frac{1}{3}$ mm. rise = 9 cubic mm.) The pairs were tested as described above with Roy's membrane or collodion film.

The second form of recording apparatus is the Differential Recording Osmometer shown in fig. 3 *a* and *b*, Plate VIII.

To the pointer of the balance (Dutch system) is attached a light horizontal brass wire, projecting forwards and having a forked extremity bent downwards. At the front of the base board of the balance is set a slender vertical spindle which at its lower bearing, after passing through a collar, rests on a polished steel plate. Rigidly attached to this spindle at its lower part, is a brass wire, passing horizontally backwards between the prongs of the fork in connection with the pointer of the balance. Any lateral movement of the pointer thus causes a rotation of the vertical spindle, and the amount of rotation

consequent upon any given deflection can be increased or diminished by sliding the bed bearing the spindle to or from the pillar of the balance, slots and screws being provided for this purpose. On the spindle is a piece of pith capable of rotation, elevation, and depression; the anterior surface of this piece of pith bears an ordinary galvanometer mirror (*M*). At a distance of 37 centimetres from the mirror is fixed a camera (*C*) with a horizontal slit in the front board, above which is placed a millimetre scale. The internal construction of this camera is very similar to that described in connection with the first recording instrument. A roll of sensitive paper (Morgan and Kidd's bromide paper), 14 ft. long by $4\frac{1}{2}$ in. wide, is made to pass behind the slit by the action of a clock, the paper being kept strained by a check weight. Behind the camera is placed either a paraffine lamp or gas jet, the light from which is screened except in front where it passes through a horizontal internally blackened tube bearing a vertical slit (*S*) at its extremity. The light after falling on the mirror (*M*) is reflected on to the slit and scale of the camera, the arrangement being similar to that used in the reflecting galvanometer.

Deflection of the balance thus causes rotation of the vertical spindle and consequent excursion of the reflected image of the slit, upon the scale and slit of the camera. The deflections of the image of the slit on the scale are proportional to the tangents of the angles of deflection of the beam of the balance and consequently to the weights in the pans. A lens not shown in the figure brings the spot of light to a focus on the scale.

In the pans of the balance are placed two small vessels (*V*) partially filled with olive oil and made to balance one another. Behind the pans are fixed a pair of discharging osmometers so that the oil discharged passes into the small vessels in the pans. It will be evident from the above description that, if equal amounts of oil are discharged by the two osmometers into the vessels in the pans, in a given time, the pointer of the balance and consequently the spot of light will remain at zero. If however, for example, the left osmometer discharges more than the right the pointer of the balance will move to the right and the spot of light to the left on the scale, and since the deflections of the spot of light are proportional to the weights, it is possible to determine by the deflection how much more oil has passed out of the left than out of the right osmometer, the deflection proportional to any given weight having been previously determined by experiment. The osmometers figured

were made double in order that the surface of membrane exposed might be increased if necessary in cases where only small pieces can be obtained, as in the case of the gastric mucous membrane of the frog; as a rule however the instrument was found to be sufficiently sensitive when only one of the two lower apertures of the osmometer was made use of, the other being closed with a paraffined cork. The degree of sensitiveness employed in practice was that giving a deflection of 13 mm. on the scale with each centigramme of difference of weight between the pans. The rate of movement of the paper in the camera was 11·5 mm. per hour. A thread soaked in oil was tied to the discharging tube of each osmometer and dipped below the surface of the oil in the small vessels to allow a continuous pasage of oil from the osmometers into the vessels. The time was marked in this apparatus by a method very similar to that described in the first instrument; a fixed mirror (*K*) reflected part of the light passing through the slit in the tube on to the slit in the camera; this light was prevented from entering the camera by a small piece of cardboard (*L*); once in every hour however the lever in connection with the hour axis of the clock causes the cardboard to be withdrawn so that the photographic paper is exposed and a black dot marking the hour appears on development.

By rotating the pith bearing the mirror on the spindle, the zero of the instrument can be set at various parts of the scale, an advantage when one only has 4½ inches of paper for recording purposes. The pair of discharging osmometers selected for use with this instrument gave, when covered with collodion film and filled with 5 % glucose solution in water, a deflection of only one millimetre in 24 hrs., the line traced being almost parallel to the base line marked by the time tracing. Both the above recording instruments were worked in a room lighted by non-actinic light.

The osmograph of Carlet[1] is not adapted for use with small pieces of membrane; in this instrument moreover the record is taken upon a smoked surface upon which in my hands the records of slow processes, as, for instance, rigor mortis, have not been all that could be desired.

Regnard[2] has recently described and figured a Diffusiograph for use with the cæcum of the sheep as a fixed membrane; in this instrument the outflow of fluid introduced by osmosis is made to raise mercury

[1] *Comptes Rendus de l'Acad. des Sciences*, t. LXXVI., 1875.
[2] *Comptes Rendus de la Société de Biologie*, Jan. 11, 1889.

in a manometer provided with float and smoked travelling surface; the instrument would not be sensitive enough for the present experiments, and the smoked surface is again I think an objection.

Experiments with the Skin of the Frog.

Matteucci and Cima[1] published in 1845 the results of a series of experiments tending to show, that the intensity of the fluid stream produced by osmosis, through a membrane freshly removed from the body, varies, according to the disposition of the surfaces of the membrane relatively to the two fluids used for experiment. The fluids employed by these observers were, on one side of the membrane water, on the other side, one of the following fluids, cane sugar solution 19° Beaumé, white of egg solution 4° B, gum arabic solution 5° B, or alcohol 34° B; the membranes employed were the skins of the frog, the eel, and the torpedo.

These membranes were either tied on to the reservoirs of Dutrochet osmometers, or were fixed vertically between the two halves of a horizontally placed cylindrical vessel, a vertical graduated tube communicating with each half of the apparatus. The membranes were supported by perforated metallic plates; the size of the perforations in these plates and the area of the surface of membrane exposed are not however given in their paper.

With fresh frog skin and cane sugar solution 19° B (20 %), these observers noticed a more rapid rise in the column of fluid in the tubes of the osmometers in which the external face of the skin was turned towards the sugar solution than in the case where the internal face was so disposed. The results with gum arabic solution and white of egg were of a similar nature. With alcohol however they found that more water passed in a given time through the skin towards the alcohol in the direction from without inwards than in the direction from within outwards. The conclusion arrived at by these authors was that in the fresh skin of the frog, the eel, and the torpedo, osmotic transference of fluid takes place more easily from within outwards than from without inwards.

Does this condition of affairs exist in the normal living skin, or is it the result of changes in the tissues of the skin brought about by the

[1] *loc. cit.*

action of the fluids employed for experiment, by pressure, or by both of these agents acting in concert?

Before attempting to give an answer to this question it will be necessary to call attention to the following facts:

(1) The aqueous solutions of sugar, gum arabic, and egg albumin used by Matteucci and Cima are rapidly fatal to the vitality of tissues removed from the body, judging from their action upon ciliated epithelium. At 10° C. I found that the movements of the cilia of cells removed from the frog's œsophagus ceased in 4 minutes when the preparation was irrigated with the cane sugar solution. In gum arabic solution 5° B they continued for 15 minutes, while in white of egg solution 4° B cessation of ciliary motion occurred in about 1 hour.

(2) Fluids under pressure filter through the skin of the frog more easily from within outwards than from without inwards.

Cima[1] caused water at a pressure of 10 c.m. of Hg to filter through frog's skin; he found that the volume passing through in 5 minutes from within outwards occupied 37 minutes in passing in the reverse direction. This easier filtration from within outwards may be due to the easier passage of fluid through the cells lining the walls of the numerous mucous glands of the skin of the frog, being in the direction in which fluid normally passes in the act of secretion by these cells.

Von Wittich[2] notices the same fact and calls attention to the separation of the superficial layers of the epidermis which occurs when pressure is applied from within outwards; he is of opinion that the filtration occurs through the openings of the goblet cells described by F. E. Schultze[3], which are said to be concerned in ecdysis. This separation of the superficial layers of the epidermis, I have frequently noticed under a pressure as low as 10 c.m. of water, after a lapse of 24 hrs., when the skin has not received adequate support.

It occurs more quickly with skin that is stale, or which has been immersed in chloroform water or solution of sulphate of strychnia (·01 %), than with perfectly fresh skin. Immersion of the fresh skin for a minute in 1 % solution of osmic acid retards the separation under pressure of the superficial layers, probably on account of the hardening action of the reagent. Pressure from within outwards then disorganises the structure of exsected skin of the frog when not supplied with

[1] *Mem. dell' Accad. di Torino*, XIII., 1853.

[2] *loc. cit.*

[3] *Arch. f. mik. Anat.* Bd. III. s. 166, 1867.

adequate support, especially when the vitality of such skin has been lowered.

In order to obtain an answer to the question stated above, the experiments of Matteucci and Cima with 20 % cane sugar in water were first repeated, and then a series of experiments were made with 5 % glucose in normal saline solution and 5 % glucose in water, in order to compare the action of a solution tending to prolong the vitality of the skin, with that of a solution which abbreviates tissue life.

In all cases the skin was perfectly fresh, the experiment commencing as a rule within 20 minutes of the death of the frog; portions of skin from corresponding parts of the body were always selected and the glucose or cane sugar solutions freshly prepared for each experiment. The osmometers were of two patterns made to work accurately in pairs by the method already detailed. In the Dutrochet osmometers the process of osmosis commenced at a pressure of 10 c.m. of the denser fluid, which with 20 % solution of cane sugar is equal to 8·59 mm. of mercury, and with 5 % solution of glucose in normal saline is equal to 7·55 mm. of mercury; the measurements of these instruments are given in an earlier part of this paper. The other pattern of instrument was the discharging osmometer described above; the oil receivers were weighed at the commencement and again at the end of each experiment, the duration of an experiment being as a rule 24 hrs. With the Dutrochet osmometers the rise during the first six hours was generally noted and finally the height to which the column of fluid had risen in 24 hrs.; occasionally the experiment was carried on for 48 hrs. The temperature was taken by a pair of thermometers, maximum and minimum, selected for use by a thermometer maker.

Series A. 20 % *Cane Sugar in Water. Dutrochet Osmometers.*

Using cane sugar in 20 % solution in water, and the Dutrochet osmometer, the results, embodied in Table (1), are in the main confirmatory of the statement of Matteucci and Cima, that there is a more rapid water stream from within outwards than from without inwards through fresh frog's skin. It is however particularly to be noted in experiments II., III., V. and VI. in this Table, that during the earlier stages of the process the stream from without inwards is the more rapid of the two, while in the later stages the stream from within outwards exceeds that in the reverse direction; in experiment I. no difference

TABLE I. FROG SKIN. DUTROCHET OSMOMETER.

Height of column of fluid above zero at end of	I		II		III		IV*		V		VI	
	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)
1st hr.	—	—	1·0	·25	1·0	·5	·5	1·5	1·0	·75	1·0	·75
2nd hr.	·5	·5	2·0	·5	2·0	·75	1·0	3·0	1·75	1·0	—	—
3rd hr.	1+	1+	3·0	·75	—	—	1·5	5·0	2·25	1·0+	1·75	1·5
4th hr.	1·5	1·5	—	—	3·5	1·0	—	—	3·0	1·25	2·25+	2·0
5th hr.	2·0	2·0	4·0	1·75	—	—	2·0	6·75	—	—	2·5	2·5
6th hr.	2·0	2·25	4·5	2·5	4·5	2·25	2·5	7·5	—	—	3·0	3·0
24th hr.	3·5	13·5	9·0	12·0	10·0	13·0	10·0	14·0	13·75	18·0	10·5	15·0
Temp. variation.	8°—11° C.		8°–12° C.		8·5°—11° C.		9°—11° C.		——		13°—15° C.	

* In this case the membranes were purposely not supported.

Fluid in osmometer, 20 % cane sugar in water.
Fluid outside osmometer, water, 300 c.c.
a = Inner surface to cane sugar. *b* = Outer surface to cane sugar.
A rise of 1 mm. = 9 cubic mm. of fluid introduced into osmometer.
Surface area of skin, 95 sq. mm. (ebonite plates).
Pressure at commencement of experiment 8·59 mm. of Hg.

could be detected in the rapidity of the rise of the column of fluid during the first five hours; while in experiment IV., in which the ebonite supports had been purposely removed, the condition of stronger water stream from within outwards appeared to hold throughout. This last experiment shows well how necessary a support is when the Dutrochet osmometer is used without attempting to equalize pressure by immersion, for though the area of membrane exposed in experiment IV. is greater than that in experiments III. and VI., yet the height to which the column of fluid rose in 24 hrs. was nearly the same in all three cases, though a quantity of fluid proportional to the surface must have passed into the three osmometers.

The explanation of the fact that the rise of the column of fluid in osmometer (*a*), Experiment IV., is throughout slower than that in osmometer (*b*), is to be found in the absence of support to the membrane; the other experiments in which support is given, show that as a rule the passage of water during the earlier stages is easier from without inwards than from within outwards; the easier filtration and the bagging of the membrane when not supported, which occurs when the inner surface of the skin is towards the sugar solution in the osmometer, accounts for the exception to the general rule present in this experiment.

It would appear then that during the first few hours post mortem the direction of easier osmotic transference of fluid through the skin of the frog is from without inwards, when 20 % solution of cane sugar in water is employed as denser fluid, and water as less dense fluid, and when a pressure of upwards of 8·59 mm. of mercury exists against the water stream; this first stage is however succeeded by a second in which the direction of easier osmotic transference is the same as that of easier filtration, namely from within outwards.

Series B. 5 % Glucose in Normal Saline and in Water.

Discharging Osmometers and Dutrochet Osmometers.

In this series a comparison was made between the action of a 5 % solution of glucose in normal saline and that of a 5 % solution of glucose in water, in order to observe the difference between the action of a fluid tending to prolong tissue life and of one tending to accelerate death.

TABLE II. SKIN OF FROG. DISCHARGING OSMOMETER. NORMAL SALINE.

1	2	3	4	5	6	7	8	9	10
No. of experiment.	Temperature variation C.	Area of skin in square mm.	Volume of fluid in outer vessel in c.c.	Duration of experiment.	Surface of skin in contact with glucose solution.	Initial weight of oil receiver in grammes.	Terminal weight of oil receiver in grammes.	Weight of oil discharged in grms.	Volume of normal saline entering osmometer in cubic mm.
I	11°—13°	95	300	24 hrs	(*a*) Inner	5·6275	5·7815	·1540	182·798
					(*b*) Outer	5·7155	5·7735	·0580	68·846
II	11·5°—12·5°	95	300	24 hrs	(*a*) Inner	5·8310	6·0090	·1780	211·286
					(*b*) Outer	5·7865	5·8905	·1040	123·448
III	10°—12°	95	300	24 hrs	(*a*) Inner	5·6900	5·8135	·1235	146·5945
					(*b*) Outer	5·7215	5·7730	·0515	61·1305
IV	10·5°—12·5°	95	300	24 hrs	(*a*) Inner	5·8510	6·0080	·1570	186·359
					(*b*) Outer	5·6680	5·7450	·0770	91·399
V	——	95	300	24 hrs	(*a*) Inner	5·8275	5·9565	·1290	153·123
					(*b*) Outer	5·7930	5·8460	·0530	62·911
VI	11°—12°	95	300	24 hrs	(*a*) Inner	5·7325	5·8710	·1385	164·3995
					(*b*) Outer	5·8820	5·9380	·0560	66·472

Fluid in osmometer, 5 % glucose in normal saline.
Fluid outside osmometer, normal saline.
·01 gramme, Olive oil = 11·87 cubic mm.
Average amount of *normal saline* passed through skin (95 sq. mm.) in 24 hrs,
(*a*) From without inwards 174·093 cubic mm.
(*b*) From within outwards 79·035 cubic mm.

TABLE III. SKIN OF FROG. DISCHARGING OSMOMETER. WATER.

1	2	3	4	5	6	7	8	9	10
No. of experiment.	Temperature variation C.	Area of skin in square mm.	Volume of fluid in outer vessel in c.c.	Duration of experiment.	Surface of skin in contact with glucose solution.	Initial weight of oil receiver in grammes.	Terminal weight of oil receiver in grammes.	Weight of oil discharged in grms.	Volume of water entering osmometer in cubic mm.
I	10°—11°	95	300	24 hrs	(*a*) Inner	5·7930	5·8790	·0860	102·082
					(*b*) Outer	5·6845	5·7545	·0700	83·09
II	9°—11·5°	95	300	24 hrs	(*a*) Inner	5·8210	5·8980	·0770	91·1399
					(*b*) Outer	5·7320	5·7960	·0640	75·5968
III	11°—12·5°	95	300	24 hrs	(*a*) Inner	5·8455	5·8965	·0510	60·537
					(*b*) Outer	5·7130	5·7845	·0715	84·8705
IV	10°—11°	95	300	24 hrs	(*a*) Inner	5·8200	5·9260	·1060	125·822
					(*b*) Outer	5·8170	5·9325	·1155	137·0985
V	11°—13°	95	300	24 hrs	(*a*) Inner	5·8900	5·9430	·0530	62·911
					(*b*) Outer	5·8425	5·9235	·0810	96·147
VI	11·5°—12·5°	95	300	24 hrs	(*a*) Inner	5·8620	5·9210	·0590	70·033
					(*b*) Outer	5·7960	5·8680	·0720	85·464

Fluid in osmometer, 5% glucose in water.
Fluid outside osmometer, distilled water.
·01 gramme, Olive oil = 11·87 cubic mm.
Average amount of *water* passed through skin (95 sq. mm.) in 24 hrs,
(*a*) From without inwards 85·420 cubic mm.
(*b*) From within outwards 93·711 cubic mm.

The experiments included in Tables (2) and (3) were made with a pair of discharging osmometers, in which the pressure is maintained constant. This method while it gives the net result of the process when pressure is constant, gives no indication of any variation, occurring from time to time, in the rapidity of passage of fluid into the osmometer.

By reference to columns 9 and 10 in these Tables, it will be seen that when the experiment is made with solutions tending to destroy tissue life, viz. water and watery solution of glucose (Table 3), the fluid stream in 24 hrs. from within outwards through fresh frog's skin, is as a rule greater than that from without inwards. If however the solution tend to prolong the vitality of the skin, as is the case when the glucose is dissolved in normal saline and normal saline used as less dense fluid (Table 2), then the stream from without inwards exceeds that in the reverse direction. It is to be noted also that in 24 hrs. the average amount of fluid passing through the fresh skin is greater when that fluid is normal saline than when it is water. The fresh and living skin is then more permeable than the dying skin, but as will be noticed later this period of diminished permeability is followed by a state in which

the stale skin becomes more permeable than the fresh. Englemann[1] in his experiments upon the electro-osmotic permeability of various substances has also noticed that skin kept some days is more permeable than that freshly removed.

It is also of importance to note that,

(1) From without inwards the fresh skin is more permeable by normal saline than by water.

The average number of cubic millimetres of normal saline and water passing in 24 hrs. for this position being,

Normal saline	Water
174·093.	85·420.

(2) From within outwards the skin is more permeable by water than by normal saline, the average number of cubic millimetres of normal saline and water passing in 24 hrs. for this position being,

Normal saline	Water
79·035.	93·711.

Tracings 2 and 3, Plate IX., are illustrative of this point.

Differences in the amounts of fluid passing in 24 hrs. through the skins of different individuals are also noticeable, and cannot be explained by variations of temperature alone. With the fresh skin of frogs which are in an enfeebled condition at the end of the breeding season, the difference between the amounts of fluid passing in the two directions, viz. from without inwards and from within outwards, is not so great as in the case of the skin of a vigorous animal. As will be noticed later the same condition is found to exist when fresh is compared with stale skin.

At constant pressure then, where error from filtration is excluded, and where solutions are employed which do not rapidly kill the tissues of the excised skin, the direction of easier osmotic transference of fluid is found to be from without inwards. The substitution of water for normal saline as a solvent of the glucose, as a rule reverses this state of affairs, because the water kills the tissues of the skin with rapidity, and as will be shown later the direction of easier osmotic transference of fluid through dead skin is in the direction of easier filtration, namely from within outwards. Cases occur however such as I. and II. in Table (3), where with watery solution of glucose the easier passage is in the same direction as with the solution in normal saline. In these cases however

[1] *loc. cit.*

it is noticeable that the volumes of fluid passing in the two directions, from without inwards and from within outwards respectively, approach nearer in magnitude than is ever the case when the fluids are normal saline and solution of glucose in normal saline, and the vigour of the tissues of the skin consequently less impaired. In such cases (I. and II. Table (3)) I imagine that the vitality of the tissues of the skin is great, and in 24 hrs. their death has not been accomplished, though life is at a low ebb. A few experiments made with the Dutrochet osmometer with watery solution of glucose and the skin in the two positions, showed sometimes a quicker rise of the column of fluid in the osmometer in which the inner surface of the skin faced the glucose solution, during the first few hours, while by 24 hrs. the heights of the columns were nearly the same; but in all cases by 48 hrs. the height of the column in the case where outer surface of skin was towards glucose, was greater than that in the other tube, in which inner surface was towards glucose.

TABLE IV. FROG SKIN. DUTROCHET OSMOMETER.

Height of column of fluid in mm. above zero at end of	I		II*		III		IV		V		VI	
	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)
1st hr.	·75	·25	—	—	1·0	·5	·75	·25	·75	·5	·75	·25
2nd hr.	1·5	·25+	·5	·25	1·75	·75	1·5	·25	1·5	1·0	1·25	·5+
3rd hr.	2·5	·5	·75	·5	—	—	2·0	·5−	2·25	1·5−	2·0	·75
4th hr.	3·0+	·75	1·0	·75	3·5	1·5	2·5	·75	3·0	1·5+	2·5	1·0
5th hr.	3·5	1·0	—	—	5·0	1·75	—	—	3·5	2·0	3·0−	1·25
6th hr.	3·5+	1·25	1·5	1·0	5·5	2·0	3·5−	1·25	—	—	3·75	1·5
24th hr.	5·0	4·5	4·0	6·5	14·0	11·5	7·0	4·0	—	—	8·5	7·0
Temp. variation.	9°—11° C.		11°—12° C.		——		8°—11·5° C.		9°—11° C.		8°—10° C.	

* Feeble frog.

Fluid in osmometer, 5 % glucose in normal saline.
Fluid outside osmometer, normal saline 300 c.c.
Surface area of skin 95 sq. mm. (Ebonite plates.)
Pressure at commencement of experiment 7·55 mm. Hg.
(*a*) = Inner surface to glucose solution.
(*b*) = Outer surface to glucose solution.
A rise of 1 mm. = 9 cubic mm. of fluid introduced into osmometer.

In Table (4) are recorded experiments with fresh frog skin, using Dutrochet osmometers, when 5 % glucose in normal saline is employed at a pressure of 7·55 mm. of mercury.

The precautions detailed at the commencement of the paper were adopted throughout.

In all cases it is seen that the direction of easier passage of fluid is from without inwards, the same condition as was observed in the experiments with the same fluids at constant pressure (Table 2). The average amount of normal saline passing from without inwards through the skin in 24 hrs. is 69·3 cubic mm., as against 60·3 cubic mm. passing in the reverse direction. That the numbers obtained by this method approach one another in magnitude so much more nearly than is the case when the experiment is conducted at zero pressure, is due to the fact that more fluid is lost by filtration at the pressure of 7·5 mm. of mercury, when the inner surface of the skin is towards the glucose solutions, than when the outer surface is so disposed, for filtration from within outwards occurs more easily than from without inwards.

The normal direction of easier osmotic transference of fluid through the living skin of the frog must then be considered to be from without inwards, while filtration under pressure takes place with greater ease in the reverse direction.

The fact noticed above that from within outwards the fresh skin is more easily traversed by water, a reagent destructive of tissue life, while from without inwards it is more permeable by normal saline, a reagent tending to prolong the vitality of tissues removed from the circulation, led me to think that some directive force is present in some tissue of the living skin of the frog tending to promote the passage of fluids from without inwards, of a nature similar to the secretive force exerted by the cells of a gland, by virtue of which the secretion is produced at a pressure greater than that of the blood.

If such an absorptive force dependent upon tissue life acted in a direction from without inwards, depression of vitality would tend to slow the passage of fluid from without inwards, but if the fluid be passing from within outwards, the same depression of vitality would, by lessening an opposing force, tend to quicken the ordinary osmotic stream. Such a condition of affairs occurs when watery solution of glucose is substituted for the solution in normal saline. The differences observed between the volumes of fluid passing in a given time through the skins of vigorous and of feeble frogs strengthened this conception, and it will be remembered that Cazeneuve and Livon[1] in their experiments with dialysis of urea through the wall of the bladder noticed differences of a somewhat similar nature.

[1] *loc. cit.*

Thinking that experiments conducted with parts of the skin of the same frog and in which a pressure existed against the osmotic stream would be more conclusive, I made the experiments recorded in Tables 5 and 6.

TABLE V. FROG SKIN. DUTROCHET OSMOMETER.

Inner surface of skin to glucose.

Height of column of fluid in mm. above zero at end of	I		II		III		IV		V	
	x	*y*	*x*	*y*	*x*	*y*	*x*	*y*	*x*	*y*
1st hr.	·75	·25	·5	·5 –	—	—	·75	·5	1·0	·75
2nd hr.	1·25	·5	1·0	·5 +	1·0 –	·25	1·0 +	·75	1·75	1·25
3rd hr.	—	—	1·5	1·0	1·25	·5	1·5	1·0	2·25	1·5
4th hr.	2·0 +	1·0	1·75	1·0 +	1·5	·5 +	—	—	—	—
5th hr.	2·5	1·0 +	2·0	1·25	1·5 +	·75	2·0	1·25	3·0	1·5 +
6th hr.	2·5 +	1·25	—	—	2·0	1·0	—	—	3·0 +	1·75
24th hr.	8·0	4·0	8·0	5·0	6·25	4·0	6·75	3·25	5·0	3·25
Temp. variation.	8·5°—10·5°		——		9°—11·5°		10°—11·5°		12°—13°	

Fluid in osmometer *x*, 5 % glucose in normal saline.
Fluid in osmometer *y*, 5 % glucose in water.
Surface area of skin, 95 sq. mm. (Ebonite plates.)
A rise of 1 mm. = 9 cubic mm. of fluid introduced into osmometer.
Pressure at commencement $x = 7{\cdot}55$ mm. Hg.
$y = 7{\cdot}52$ mm. Hg.

TABLE VI. FROG SKIN. DUTROCHET OSMOMETER.

Outer surface of skin to glucose.

Height of column of fluid in mm. above zero at end of	I		II		III*		IV		V	
	x	*y*	*x*	*y*	*x*	*y*	*x*	*y*	*x*	*y*
1st hr.	0	1·0	·25	·5	0	1·0	0	·75	+	1·5
2nd hr.	0	2·0	·25	·75	0	1·25	·25	1·25	·5	2·0
3rd hr.	·5	2·5	·5	1·0	0	1·75	·25 +	2·0	·75	2·5
4th hr.	—	—	—	—	0	2·0	·5 +	2·75	1·0	2·5 +
5th hr.	·75	3·25	·75	1·25	—	—	1·0	3·5	—	—
6th hr.	1·0 +	3·5	·75	1·5	·25	2·5	1·5	3·75	1·25	3·0
24th hr.	6·0	6·0	1·75	2·5	6·0	6·5	6·5	7·25	2·75	5·5
Temp. variation.	——		9°—11°		10°—12°		9°—11°		8°—11°	

* By 48 hrs. $x = 8{\cdot}0$ $y = 13{\cdot}0$

Fluid in osmometer *x*, 5 % glucose in normal saline.
Fluid in osmometer *y*, 5 % glucose in water.
Surface area of skin, 95 sq. mm. (Ebonite plates.)
A rise of 1 mm. = 9 cubic mm. of fluid introduced into osmometer.
Pressure at commencement $x = 7{\cdot}55$ mm. Hg.
$y = 7{\cdot}52$ mm. Hg.

In Table (5) where a solution of glucose in normal saline is used, the average rise in 24 hrs. is 6·8 mm., when the inner surface of the skin is towards the glucose solution; when however, the skin being in the same position, the solution of glucose is made with water, the average rise is only 3·9 mm.

Reversing the position of the skin (Table 6) so that the outer surface is towards the solution of glucose, it is found, that where normal saline is used, the rise in 24 hrs. is, on an average, 4·51 mm., as against 5·28 mm., when a watery solution is employed.

The contrast is even better demonstrated during the earlier stages of each experiment. Thus if reference be made to the height of the columns of fluid in the two osmometers at the 5th or 6th hour of the experiment, it will be seen that the differences are more marked than at the 24th hour. This one would naturally expect to be the case if the difference be dependent upon some form of protoplasmic activity, an activity continually on the wane in the tissue removed from the circulation.

Series C. Experiments at various periods post mortem.

Discharging Osmometers. 5 % Glucose in Normal Saline.

In these experiments the process of death of the tissues of the skin was observed more closely, in the hopes of finding the point at which this apparent directive force present in the living skin ceases to exist. For this purpose the skins of the largest frogs that could be obtained were kept in normal saline solution for various lengths of time after the death of the animal; portions from corresponding parts of the body were then fixed on to a pair of discharging osmometers, the inner surface of the skin facing the glucose solution in one instrument while in the other the outer surface was so arranged.

Since in the skin kept in normal saline a separation of the superficial layers of the epidermis takes place after a lapse of about 72 hrs., it was considered advisable to first determine the effect, upon the phenomena of osmotic transference of fluid through the skin, of the removal of these layers from the fresh skin. For this purpose, the outer surface of the skin, after removal, was scraped with a blunt scalpel, and the scraped portions tied on to Dutrochet osmometers, in which were placed glucose dissolved in either normal saline or water. The amount of material removed by the scraping was determined by hardening

pieces of the skin in Müller's fluid and examining sections with the microscope. In all cases only the first and second layer of cells of the epidermis were found to have been removed, the lower layers appearing, as regards their structure, to be uninjured. The few experiments made are placed in Table 7.

TABLE VII. SCRAPED FRESH FROG'S SKIN. DUTROCHET OSMOMETER.

Height of column of fluid in mm. above zero at end of	NORMAL SALINE.				WATER.			
	1		2		1		2	
	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)	(*a*)	(*b*)
1st hr.	1·5	·5+	1·0	0	·75	2·0	·5	1·5
2nd hr.	2·0	1·0	2·0	+	1·5	3·25	1·0	2·5
3rd hr.	2·5	1·25	2·5	·5	1·75	4·0	1·0+	2·75
4th hr.	2·75	1·25+	2·75	·75	2·0	4·25	1·5	3·0
5th hr.	3·0+	1·5	3·0	1·0	—	—	1·75	3·5
6th hr.	3·25	1·5+	—	—	2·75	4·75	1·75	3·5+
24th hr.	5·5	4·0	6·0	6·0	5·0	7·0	4·0	6·0+
Temp. variation.	5°—11° C.		5°—8° C.		8°—12° C.		8·5°—10·5° C.	

Fluid in osmometer, 5 % glucose in normal saline or water.
Fluid outside osmometer, normal saline or water 300 c.c.
(*a*) = Inner surface to glucose.
(*b*) = Outer surface to glucose.
A rise of 1 mm. = 9 cubic mm. of fluid introduced into osmometer.
Surface area of skin = 95 sq. mm. (Ebonite plates.)
Pressure at commencement of experiment
if normal saline used 7·55 mm. Hg.
if water used 7·52 mm. Hg.

A comparison of the two experiments in this table, in which normal saline was used, with those in table (4), shows that the removal of the superficial layers of the epidermis has little if any effect. With watery solution of glucose however it is to be noted that from the very first the fluid stream from within outwards exceeds that from without inwards, and to a very marked degree. The explanation of this fact appears to be as follows: in the first place filtration will take place more easily where inner surface is towards the pressure, now that the superficial layers have been removed; in the second place by removal of the superficial layers, which are more or less corneous in texture, easier access is given for the fluid to the parts beneath. If this fluid be one tending to prolong tissue life, this easier admittance

will be of little moment, for the duration of life in the more internal parts of the skin will not be affected; such is the state of affairs where normal saline is used. If on the other hand the fluid be a depressant of tissue life, the case when water is used, the removal of the outer corneous layer will, by allowing the water to come in contact with the inner parts of the epidermis, speedily bring about their death, and if the existence of an easier passage of fluid in the direction from without inwards be dependent upon the life of the tissues of the skin, the direction of easier osmotic transference will no longer be from without inwards, but from within outwards, as is the case in dead skin. It will be remembered that in the experiments with fresh intact skin with 20 % aqueous solution of cane sugar, quoted in Table 1, an early stage was noted where the direction of easier transference of fluid was from without inwards, but that this stage was followed by a second, in which the reverse held good; this condition was considered to be due to the fact that, during the first stage, the skin was capable of holding its own against the depressing action of the fluids; when death of the tissues of the skin occurred the second stage supervened, namely, an easier transference of fluid from within outwards.

In the scraped skin when water is employed, no first stage is observable, for the destructive agent is able to get at the tissues almost immediately and bring about their death. That the effect is not due to the mechanical injury of the skin by the scraping, is shown by the normal course taken by the experiments with normal saline.

Having thus determined that, where a normal saline solution of glucose is used, the separation of the superficial layers of the epidermis may be neglected, I proceeded to study the phenomena of the passage of fluid through the frog's skin at various periods post mortem. For this purpose the differential recording osmometer described above was employed. In all experiments the skin on the right osmometer was placed with its inner surface towards the solution of glucose in normal saline contained in the reservoir of the apparatus, while on the left osmometer the outer surface was in contact with the glucose solution. Any deflection of the spot of light towards the right (time tracing side of the record) indicated that more fluid had passed through the skin on the right osmometer in the direction from without inwards, than through the skin on the left osmometer in the direction from within outwards. The surface of skin exposed was 95 sq. mm. and 200 c.c. of salt solution were placed in the outer vessel belonging to each osmo-

meter. With large frogs it was found possible to obtain 4 pairs of pieces of skin of the area used. Thus by removing the skin from a large frog and placing it in normal saline, experiments with parts of the skin of the same animal could be conducted as four separate periods post mortem.

At the degree of sensitiveness at which the apparatus was worked, a deflection of 1 mm. towards the time tracing indicated that ·91 cubic mm. more fluid had passed through the skin on the right osmometer where the inner surface was towards the glucose solution, than through the skin on the left osmometer where the outer surface was towards the glucose solution. In other words a deflection of 1 mm. towards the time tracing indicated that ·91 cub. mm. more fluid had passed from without inwards than from within outwards; the same deflection away from the time tracing of course indicates the reverse of this. Experimenting in this manner, it was found that the longer the period that had elapsed since the death of the animal, the smaller was the number representing the excess of the quantity of fluid passing from without inwards over that passing from within outwards, until a stage was reached at which the amount passing in the two directions was the same. Up to this stage the amount of fluid passing from without inwards was always greater than the amount passing from within outwards; but after the stage at which equality was established, a third period commenced in which the amount passing from within outwards was in excess of that passing from without inwards, and again the longer the time which had elapsed since the stage of equality, the greater was the number representing the excess of the quantity of fluid passing from within outwards over that passing in the reverse direction. Thus in the exsected skin three periods post mortem can be distinguished.

A first period during which the quantity of fluid passing in the direction from without inwards is in exeess of the quantity passing from within outwards. As time passes during this period, the quantities passing in the two directions gradually approach one another in magnitude until the second period is reached.

During the second period the differential instrument traces a straight line, for equal amounts of fluid are passing into the osmometers discharging into the two sides of the balance.

In the third period the quantity of fluid passing from within outwards is in excess of the quantity passing from without inwards,

and the number representing this excess increases with the lapse of time post mortem.

As regards the time of advent and duration of these periods, it was found that there was a distinct connection between the vital condition of the skin and the duration of the first period and advent of the second. With the skin of a vigorous animal the first period may continue till 70 to 80 hrs. post mortem when the skin has been kept in normal saline after removal. With the skin of frogs enfeebled at the end of the breeding season, or when the skin is kept at a temperature of 25° to 30° C., its duration may be as short as till 24 hrs. post mortem. Again if the exsected skin be kept in a solution of glucose in normal saline, instead of in normal saline, the duration of the first period will be shortened.

The second period is always of short duration, indeed it represents merely the transition from the state of affairs existing in the first period to that in the third; its duration is also associated with the vigorous or feeble condition of the animal. In the skin of a vigorous animal kept in normal saline, it may last for 4 or 5 hours, while any influences tending to lessen the vitality of the tissues will cut it down to about an hour.

The third period I have followed till 125 hours post mortem, when I still found that the permeability of the skin from within outwards was on the increase.

The experiments were conducted at temperatures varying from 8—13° C.

Tracing 4, Plate IX., illustrates the above points.

Tracing 5, Plate IX., was taken with 190 sq. mm. of skin, instead of 95 sq. mm. as is the case in the other records; it shows the end of the first stage, the second, and the commencement of the third.

Referring back to the results embodied in Table 3, it will be seen that the effect of treating the skin with a watery solution of glucose is to produce a condition of easier osmotic transference of fluid from within outwards than in the reverse direction. The experiments just detailed show that a similar effect can be produced by allowing the skin to remain about 3 days in normal saline before employment for experiment. Watery solution of glucose then merely brings on more quickly a change which will occur if the tissues of the skin be left to die in normal saline, this change being the loss of the function of the skin of the frog by virtue of which an easier passage of fluid from without inwards than from within outwards is effected.

Turning now to post mortem variations in the permeability of the skin in the direction from without inwards, it was found when corresponding parts of the skin of the same animal were used for experiment, that two stages could be distinguished after removal of the skin from the body. During the first stage, lasting some 24 hrs. post mortem, the fresh skin was found to be more permeable from without inwards than the stale skin; this stage was however succeeded by a second in which the reverse obtained. The same condition may be observed when the permeability of fresh skin to normal saline and to water respectively is tested; at first the skin is more permeable to normal saline than to water in the direction from without inwards; if however the experiment be prolonged or especially if the temperature be high, a second stage occurs during which the skin is more permeable to water than to normal saline. When however the degrees of permeability in the direction from within outwards are examined at various times post mortem, it is found that from the time of removal of the skin from the animal the permeability in this direction increases, slowly at first, but more rapidly as time passes.

Experiments illustrative of these facts are placed in Tables 8 and 9.

TABLE VIII. SKIN OF FROG. FRESH v. STALE. DISCHARGING OSMOMETER.

Inner surface to glucose solution.

Number of experiment.	Temp. variation C.	Area of skin in sq. mm.	Volume of fluid in outer vessel in c.c.			Discharge of oil in grammes.	Volume of normal saline entering osmometer in cubic mm.
I	18°—20°	95	300	Fresh	In 2 hrs	·010	11·87
					In 24 hrs	·1010	119·887
				2 hrs stale	In 2 hrs	0	0
					In 24 hrs	·045	53·415
II	17°—19°	95	300	Fresh	In 3 hrs	·0190	22·553
					In 24 hrs	·1205	143·0335
				3 hrs stale	In 3 hrs	·0150	17·805
					In 24 hrs	·1180	140·066
III	18°—21°	95	300	Fresh	—	—	—
					In 24 hrs	·1855	220·1885
				22 hrs stale	—	—	—
					In 24 hrs	·2905	344·8235
IV	18·5°—20°	95	300	Fresh	In 5 hrs	·0255	30·2685
					In 21 hrs	·1255	149·9685
				24 hrs stale	In 5 hrs	·0545	64·6915
					In 21 hrs	·1680	199·416

Fluid in osmometer, 5 % glucose in normal saline.
Fluid outside osmometer, normal saline.
·01 gramme olive oil = 11·87 cubic mm.

TABLE IX. SKIN OF FROG. FRESH v. STALE. DISCHARGING OSMOMETER.

Outer surface to glucose solution.

Number of experiment.	Temp. variation C.	Area of skin in sq. mm.	Volume of fluid in outer vessel in c.c.			Discharge of oil in grammes.	Volume of normal saline entering osmometer in cubic mm.
I	—	95	300	Fresh	In 3 hrs	·0110	13·057
					In 24 hrs	·0710	84·277
				3 hrs stale	In 3 hrs	·0190	22·553
					In 24 hrs	·0990	117·513
II	17°—20°	95	300	Fresh	In 3 hrs	·0085	10·0895
					In 24 hrs	·0880	104·456
				3 hrs stale	In 3 hrs	·0110	13·057
					In 24 hrs	·0960	113·952
III	18°—20°	95	300	Fresh	—	—	—
					In 24 hrs	·1030	122·261
				24 hrs stale	—	—	—
					In 24 hrs	·1375	163·2125
IV	18·5°—21°	95	300	Fresh	In 2 hrs	·0050	5·935
					In 24 hrs	·0900	106·83
				24 hrs stale	In 2 hrs	·0160	18·992
					In 24 hrs	·1230	146·001

Fluid in osmometer, 5% glucose in normal saline.
Fluid outside osmometer, normal saline.

·01 gramme olive oil = 11·87 cubic mm.

While then in the direction from without inwards a stage exists, immediately after the death of the animal and lasting some 24 hrs., during which the skin is more permeable than skin which has been removed for more than 24 hrs. and kept in normal saline solution, in the opposite direction, *i.e.* from within outwards, no such condition is observed; in the latter case, with the lapse of time post mortem, permeability increases, and no first stage of diminution of permeability appears to exist.

If the above facts be considered in conjunction with those observed when contrasting the permeability of the skin in the two directions at various periods post mortem, it will be seen that during the time I have called the first period, permeability from within outwards must increase faster than permeability from without inwards, and must finally, in the third period, outstrip it altogether; for it has been seen that the amounts of fluid passing through the skin in the two directions gradually approach one another in magnitude, until during the second period they are equal, while in the third the direction of easier osmotic transference of fluid is the reverse of that which is observed in the living and fresh skin.

During the first 24 hrs. or so of the first period the marked excess of the amount of fluid passing from without inwards over that passing from within outwards, must, one would think, have its causation in some form of protoplasmic activity, for as will be seen in the next section, the amount of fluid passing from without inwards through the fresh and living skin can be increased by the action of a stimulant. A stage next occurs during which permeability from without inwards is diminished, while in the opposite direction it is steadily increasing, and the flattening of the curve traced by the differential instrument at this stage is an indication of this condition, vide (*b*) Tracing 4, Plate IX. So far it would appear that the permeability of the skin has been associated with some active force acting from without inwards, such force being against the osmotic stream when the fluid is passing from within outwards. After this stage in the first period, the increase of permeability would appear to be a matter of post mortem alteration of tissue, and, as one would expect, the greater permeability in the direction of easier filtration, which up to this time has been masked by protoplasmic action, now comes into play, and the amount of fluid passing from within outwards after gradually gaining on the amount passing from without inwards, at first balances it in the second period, whilst in the third it is in excess.

This latter series of experiments was conducted at temperatures varying from 17—21° C., and consequently death of the tissues of the exsected skin must have occurred more quickly than in those in the first part of this section, where the temperature variation was but 8°—13° C.; thus it is possible that a change in the direction of easier osmotic transfer occurring at 72 hrs. post mortem at the lower, may have taken place by 24 hrs. at the higher temperature, if such change were dependent upon tissue life.

Series D. Action of Stimulants and Depressants.

The natural course to have followed at this stage of the investigation would have been to have observed the effect of removal of the epidermis *in toto* upon the phenomena of osmotic transference of fluid through the living skin. Total removal of the epidermis of the skin of the frog is however not practicable without disorganising its structure to such an extent that it is useless for experiment. It was therefore decided to observe the action of a depressant and a stimulant upon the passage of fluid through the living skin.

The action of water has already been referred to at some length, an action which must be considered to be depressant in nature, and not the result of the occurrence of water rigor; for if one supposed the diminution in the amount of fluid passing from without inwards, when water is substituted for normal saline, to be due to rigor of the tissues and consequent decrease of permeability, the same hypothesis would not hold good when the osmotic stream is made to pass from within outwards, in which direction the stream of water exceeds that of normal saline.

If the addition of a reagent which depresses tissue life, to the fluids used for experiment, is capable of diminishing the amount of fluid passing from without inwards through the living skin, while one of a stimulating nature causes augmentation, then it is at least probable that we are dealing with some force in addition to osmosis, and which is dependent upon the activity of the protoplasm of the tissues of the skin.

In the experiments in this series normal saline and 5 % glucose in normal saline were used as fluids, the reagent being added to the same amount to the fluid in the osmometer, and the fluid in the outer vessel. The osmometers were in pairs, one being used to observe the effect of the addition of the stimulant or depressant, while the other served as a control. To avoid physical error the solutions were tested in a pair of osmometers covered by boiled egg-shell membrane to ascertain whether the addition of the reagents to the amount used in the experiments influenced the process of osmosis through a dead membrane.

The first reagent whose action was tested was morphia bimeconate added to the extent of ·1 % to the solutions. No effect on the transference of fluid through the skin was observed, the amount of oil discharged by a pair of discharging osmometers and the height to which the columns of fluid rose in a pair of Dutrochet osmometers being the same.

Electrical stimulation was next tried, a platinum electrode being fused through the wall of the osmometer and another being placed in the outer vessel. An inductorium with Helmholtz modification, and whose current was reversed every five minutes, was employed, a Daniell cell being in the primary circuit. Though the current was reversed as frequently as stated I could not convince myself of freedom from error due to kataphoric action and consequently abandoned this method.

Chloroform was next employed for experiment. In the first experiments which were made with Dutrochet osmometers without support

to the membrane, it appeared that for some hours all osmotic transference of fluid through the skin was stopped when chloroform was added to the fluids, by shaking and subsequent filtration. Subsequent experiments by better methods have shown me that I was in error to some extent, from the occurrence of bagging of the skin under pressure. By employing discharging osmometers, or even Dutrochet osmometers if the skin be well supported, it is seen that if the skin be placed with its inner surface towards the glucose solution the addition of chloroform causes a diminution in the amount of fluid passing in a given time from without inwards, though never an actual cessation of osmotic transference of fluid.

The chloroform was well shaken with the solution of glucose in normal saline, and the fluid after filtration introduced into a discharging osmometer; the normal saline solution in the outer vessel was prepared in a similar manner, a globule of chloroform being allowed to remain at the bottom of the vessel. In the control osmometer was placed some of the same solution of glucose but without the addition of chloroform,

TABLE X. SKIN OF FROG. DISCHARGING OSMOMETER. ACTION OF CHLOROFORM.

Inner surface of skin towards glucose solution.

1	2	3	4	5	6	7	8	9	10
No. of experiment.	Temperature variation C.	Area of skin in square mm.	Volume of fluid in outer vessel in c.c.	Duration of experiment.		Initial weight of oil receiver in grammes.	Terminal weight of oil receiver in grammes.	Weight of oil discharged in grms.	Volume of normal saline entering osmometer in cubic mm.
I.	16°—18°	95	300	24 hrs	No chloroform	5·7300	5·8500	·1200	142·44
					Chloroform	5·5165	5·6065	·0900	106·83
II.	15°—16·5°	95	300	24 hrs	No chloroform	5·3430	5·5150	·1720	204·164
					Chloroform	5·6590	5·7300	·0710	84·277
III.	20°—23°	95	300	24 hrs	No chloroform	5·8590	5·9625	·1035	122·8545
					Chloroform	5·6070	5·7135	·1065	126·4155
IV.	15·5°—17°	95	300	24 hrs	No chloroform	5·4950	5·6550	·1600	189·92
					Chloroform	5·4620	5·5640	·1020	121·074
V.	16°—18°	95	300	24 hrs	No chloroform	5·7060	5·8440	·1380	163·806
					Chloroform	5·7520	5·8475	·0955	113·3585

Average amount of normal saline passed through skin (95 *sq. mm.*) *in* 24 *hrs.*

No chloroform in normal saline, 164·6569 cubic mm.

Chloroform in normal saline, 110·391 cubic mm.

Fluid in osmometer, 5% glucose in normal saline + chloroform or 5% glucose in normal saline.

Fluid outside osmometer, normal saline + chloroform or normal saline.

·01 gramme olive oil = 11·87 cubic mm.

TABLE XI. SKIN OF FROG. DISCHARGING OSMOMETER. ACTION OF CHLOROFORM.

Outer surface of skin towards glucose solution.

1 No. of experiment.	2 Temp. variation C.	3 Area of skin in sq. mm.	4 Volume of fluid in outer vessel in c.c.	5 Duration of experiment.	6	7 Initial weight of oil receiver in grammes.	8 Terminal weight of oil receiver in grammes.	9 Weight of oil discharged in grms.	10 Volume of normal saline entering osmometer in cubic mm.
I	15·5°—18°	95	300	24 hrs	No chloroform	5·5630	5·6325	·0695	82·4965
					Chloroform	5·8125	5·9585	·1460	173·302
II	16°—18°	95	300	24 hrs	No chloroform	5·7915	5·8655	·0740	87·838
					Chloroform	5·3860	5·5440	·1580	187·546
III	17·5°—20°	95	300	24 hrs	No chloroform	5·6380	5·7005	·0625	74·1875
					Chloroform	5·4800	5·6100	·1300	154·31
IV	12°—14°	95	300	24 hrs	No chloroform	5·5920	5·6415	·0495	58·7565
					Chloroform	5·6230	5·7310	·1080	128·196
V	18·5°—21°	95	300	24 hrs	No chloroform	5·8410	5·9110	·0700	83·09
					Chloroform	5·7555	5·8835	·1280	151·936

Average amount of normal saline passed through skin (95 *sq. mm.*) *in* 24 *hrs.*

No chloroform in normal saline, 77·2737 cubic mm.

Chloroform in normal saline, 159·058 cubic mm.

Fluid in osmometer, 5 % glucose in normal saline + chloroform, or 5 % glucose in normal saline.

Fluid outside osmometer, normal saline + chloroform or normal saline.

·01 gramme olive oil = 11·87 cubic mm.

normal saline being in the outer vessel. Preliminary experiments with boiled egg-shell membrane showed that the addition of the chloroform had no effect upon the ordinary process of osmosis.

A reference to Table 10 shows that where the inner surface of the skin is towards the glucose solution and fluid consequently passing from without inwards, the addition of chloroform as a rule causes a diminution in the amount of normal saline passed in 24 hrs., the average numbers in this table being about 164 cubic mm. through 95 sq. mm. of skin where no chloroform is present, as against 110 cubic mm. where chloroform has been added.

To determine whether this diminution in the quantity of fluid passed was due to rigor it was necessary to experiment with pieces of skin in which the outer surface was towards the glucose solution and fluid consequently passing from within outwards.

In Table 11 will be found the experiments with the skin placed so that the outer surface is towards the glucose solution. In this position

it is seen that the addition of chloroform increases the amount of fluid passing from within outwards in 24 hrs. On an average during 24 hrs. only 77 cubic mm. of normal saline passed from within outwards where no chloroform was present in the solutions, while in the cases where the reagent had been added 159 cubic mm. passed.

The addition of chloroform then to the solutions while it diminishes the amount of fluid passing through the fresh skin from without inwards, augments the stream in the opposite direction.

The above is the general rule. Exceptions occur however when the temperature is high (20° to 28° C.). In these cases the skin tissues die more quickly than at lower temperatures and as has been seen above the dead skin is more permeable than the living. Thus the diminution of permeability present in the earlier stages gives place by 24 hrs. to the increase of permeability concurrent with the death of the skin. An example of this is seen in Experiment III. Table 10.

Tracings of the action of chloroform are reproduced in numbers 6 and 7, Plate X.

Having failed through imperfection of method to obtain reliable results by electrical stimulation of the skin, the action of ethyl alcohol as a stimulant was tried.

In the first experiments the reagent was added to the extent of 1 % with results similar to those obtained with chloroform; evidently this solution was too strong and acted as a depressant.

By reducing the alcohol to ·1 % however a stimulant action was obtained. The results of these experiments are placed in Tables 12 and 13.

In Table 12, in the experiments in which the inner surface of the skin is placed towards the glucose solution, the addition of alcohol to the extent of ·1 % is found to increase the amount of normal saline passed through the fresh skin in 24 hrs., the average numbers being roughly 175 cubic mm. of normal saline in 24 hrs. when alcohol is present as against 110 cubic mm. when it is absent.

In Table 13, in the experiments in which the outer surface of the skin is towards the glucose solution and fluid passing consequently from within outwards, it will be seen that addition of alcohol diminishes the amount of normal saline passed in 24 hrs. Thus when alcohol is present to ·1 %, 58 cubic mm. passed in 24 hrs. through 95 sq. mm. of skin, on an average, while with normal saline only and glucose in normal saline 97 cubic mm. is the average number.

TABLE XII. SKIN OF FROG. DISCHARGING OSMOMETER. ACTION OF ·1% ALCOHOL.

Inner surface of skin towards glucose solution.

1 No. of experiment.	2 Temp. variation C.	3 Area of skin in sq. mm.	4 Volume of fluid in outer vessel in c.c.	5 Duration of experiment.	6	7 Initial weight of oil receiver in grammes.	8 Terminal weight of oil receiver in grammes.	9 Weight of oil discharged in grms.	10 Volume of normal saline entering osmometer in cubic mm.
I	——	95	300	24 hrs	No alcohol	5·1900	5·3160	·1260	149·562
					Alcohol ·1%	5·6945	5·8965	·2020	239·774
II	10·5°—13°	95	300	24 hrs	No alcohol	5·5440	5·6140	·0700	83·09
					Alcohol ·1%	5·8715	5·9780	·1070	127·009
III	14°—17°	95	300	24 hrs	No alcohol	5·5010	5·5870	·0860	102·082
					Alcohol ·1%	5·6320	5·7740	·1425	169·1475
IV	14·5°—18°	95	300	24 hrs	No alcohol	5·3150	5·4175	·1025	121·6675
					Alcohol ·1%	5·2240	5·3810	·1570	186·359
V	16°—18·5°	95	300	24 hrs	No alcohol	5·4900	5·5725	·0825	97·9275
					Alcohol ·1%	5·6170	5·7460	·1290	153·123

Average amount of normal saline passed through skin (95 sq. mm.) in 24 hrs.

No alcohol 110·8658 cubic mm.
Alcohol ·1% 175·0825 cubic mm.

Fluid in osmometer, 5% glucose in normal saline + ·1% alcohol, or 5% glucose in normal saline.

Fluid outside osmometer, normal saline + ·1% alcohol or normal saline.

·01 gramme olive oil = 11·87 cubic mm.

TABLE XIII. SKIN OF FROG. DISCHARGING OSMOMETER. ACTION OF ALCOHOL ·1%.

Outer surface of skin towards glucose solution.

1 No. of experiment.	2 Temp. variation C.	3 Area of skin in sq. mm.	4 Volume of fluid in outer vessel in c.c.	5 Duration of experiment.	6	7 Initial weight of oil receiver in grammes.	8 Terminal weight of oil receiver in grammes.	9 Weight of oil discharged in grms.	10 Volume of normal saline entering osmometer in cubic mm.
I	16°—19°	95	300	24 hrs	No alcohol	5·0780	5·2040	·1260	148·662
					Alcohol ·1%	5·6315	5·7380	·1065	126·4155
II	17°—19°	95	300	24 hrs	No alcohol	5·3255	5·4200	·0945	112·1715
					Alcohol ·1%	5·8965	5·9195	·0230	27·301
III	——	95	300	24 hrs	No alcohol	5·2900	5·3645	·0745	88·4315
					Alcohol ·1%	5·5280	5·5900	·0620	73·594
IV	13·5°—17°	95	300	24 hrs	No alcohol	5·5430	5·5995	·0565	67·0655
					Alcohol ·1%	5·4825	5·5045	·0220	26·114
V	16°—18·5°	95	300	34 hrs	No alcohol	5·6470	5·7080	·0610	72·407
					Alcohol ·1%	5·4220	5·4535	·0315	37·3905

Average amount of normal saline passed through skin (95 sq. mm.) in 24 hrs.

No alcohol 97·7475 cubic mm.
Alcohol 58·1630 cubic mm.

Fluid in osmometer, 5% glucose in normal saline + ·1% alcohol, or 5% glucose in normal saline.

Fluid outside osmometer, normal saline + ·1% alcohol or normal saline.

·01 gramme olive oil = 11·87 cubic mm.

With boiled egg-shell membrane and with skin which had been kept 24 hrs. in normal saline after removal, I found the addition of alcohol to ·1 % to be without effect.

The addition of alcohol to ·1 % then, while it increases the amount of normal saline passing through the fresh skin from without inwards, diminishes the amount passing from within outwards. Tracings 8 and 9, Plate X., show the action of alcohol.

Tappeiner[1] has noticed that in living cats the addition of alcohol to solutions of strychnia introduced into the stomach hastens the absorption of the drug, though from the strength of alcohol employed (nearly 25 %) the result is more probably due to vascular change than to any stimulation of absorptive function of epithelium.

Conclusion.

It has been seen that the normal direction of easier osmotic transference of fluid through the fresh and living skin of the frog is in the direction from without inwards, and not from within outwards, as the experiments of Matteucci and Cima seemed to show: filtration, bagging of membrane under pressure, and the use of fluids which tended to depress tissue life, seem to have been the causes which led these observers into error.

This difference of permeability in the two directions would appear, during the earlier stages post mortem, to be dependent more upon physiological function than upon anatomical structure. Experiments have shown that there is a close connection between the vital condition of the skin and the manifestation of its absorptive power.

Reagents tending to depress tissue life, such as water and chloroform, diminish, for some time after the death of the animal, the amount of fluid passed through the fresh and still living skin, in the direction from without inwards; while on the other hand such reagents increase the quantity of fluid passed by osmotic action from within outwards. Moderate stimulation by alcohol has been seen to increase the amount of fluid passed from without inwards, while that passed from within outwards is diminished. If the skin has been allowed to remain 24 hrs. or so in normal saline after removal the above reagents fail to affect the osmotic transfer.

When skin is employed for experiment at various times after

[1] *Zeitsch. f. Biol.* XVI. p. 497, 1880.

the death of the frog, it is seen that, while from within outwards permeability increases, slowly at first and then more quickly, in the reverse direction, namely from without inwards, permeability first diminishes and then again increases, so that it exceeds that of the fresh skin in the same direction, though in the opposite direction it is less than that of a piece at the same period post mortem. The above facts seem to favour the assumption, that in the living skin of the frog there is at work some form of absorptive force dependent upon protoplasmic activity and exerted in a direction from the external, towards the internal surface.

If we cause a stream of fluid to pass by osmosis, through the skin in a direction from without inwards, so long as the skin is alive the stream will be aided by the absorptive function of the tissues of the skin, and will be capable of augmentation by stimulation, and diminution by depression, of tissue life. If, on the other hand, it is arranged that the osmotic stream pass from within outwards, the absorptive force acting in the reverse direction will be in opposition, and while a stimulant will diminish, a depressant will increase, the total amount of fluid passed in a given time.

A reference to Plate XI. may render the above conception more clear.

In this figure the major diffusion current is in all cases to be taken as in the direction of the arrows to the right of each cut. For the sake of clearness the epithelium is represented as a single layer.

In this diagram the absorptive force has been located in the cells of the epidermis. Those who have read this paper will have seen that there is no definite evidence for such location, for it is impossible to totally remove the epithelial layers of the skin, without disorganising the structure to such an extent, that it becomes useless for experiment. When however one considers the structure of the skin of the frog, it will be seen that if such an absorptive force as I have imagined, dependent on tissue life, be present in the living skin, its most probable seat is in the epithelial layers; for since the force will act from without inwards, it cannot be dependent upon the cells of the skin glands, which would exert, in the act of secretion, a force acting from within outwards, and obviously the connective and muscular parts cannot be considered as active agents in this connection.

One would expect, if the above assumption be correct, that the absorptive force persisting for some hours post mortem would enable the skin to transfer fluid from without inwards without the aid of

osmosis. A few experiments with reference to this point have been made with discharging osmometers filled with normal saline closed by fresh skin and immersed in normal saline, a control osmometer closed with part of the same skin but in the reverse position being employed at the same time. In three cases I have seen oil discharged from the instrument in which the inner surface of the skin was towards the reservoir, though none passed from the control instrument where the skin was in the reverse or unfavourable position, the temperature variation affecting both osmometers alike. The amounts of oil discharged indicated a passage of 5·9 cubic mm. of normal saline, on an average, in the direction from without inwards, through 95 sq. mm. of skin in 48 hrs. Such experiments in conjunction with stimulants and depressants will greatly assist in elucidating the matter under consideration. If it be granted that an absorptive force is present and that it is dependent upon epithelial activity, it is still necessary to account for the fact that the ultimate result of allowing the skin to grow stale in normal saline, is to render it more permeable than it was when fresh, though this condition is preceded by one in which permeability is diminished; and again it must be borne in mind that the post mortem increase of permeability is greater from within outwards than in the reverse direction. This latter fact would appear to be dependent upon the fact that filtration occurs more easily from within outwards, whether from this being the direction in which fluids pass through the cells of skin glands in the act of secretion or from other causes.

The general increase of permeability post mortem would appear to be due to disorganization of tissue, and that fluid can pass through the dead skin with greater facility from within outwards than in the reverse direction, is probably the result of the anatomical structure of the skin with its saccular glands opening to the exterior, and perhaps also to the fact that less resistance is met in the gland cells themselves in the direction in which secreted fluid is wont to pass.

By rapidly killing the tissues of the exseeted skin with such a drug as digitaline, the passage from the state of diminished permeability from without inwards, due to the depressant action of the drug, to the state of increased permeability due to death of the tissues and commencing disintegration, may be easily observed.

A tracing illustrative of this is reproduced in number 10, Plate X.

Obviously much remains to be done to clear up the matter more

fully, and it is my intention to pursue the subject further, especially with reference to the mucosæ of the alimentary canal.

So far as the skin of the frog is concerned the following conclusions seem warranted—

1. The normal direction of easier osmotic transference of fluid through the living skin of the frog is in the direction from the outer towards the inner surface.

2. The transference of fluid through the skin in the above direction is intimately associated with the physiological condition of its tissues; conditions or agents tending to depress vitality diminish the transfer in the normal direction, while stimulants give rise to augmentation.

3. The cause of the easier transference of fluid from the outer towards the inner surface, is probably to be found in the existence of an absorptive force dependent on protoplasmic activity, and comparable to the secretive force of the gland cell.

4. In consequence of the absorptive force acting from without inwards, an alteration of the relations of the surfaces of the skin to the two fluids used in an osmosis experiment modifies the rapidity of the transfer of fluid from one to the other side of the membrane, according as the force exerted by the living tissues is with or against the osmotic stream.

[The expenses of this research were in part defrayed by a grant from the British Medical Association.]

DESCRIPTION OF TRACINGS.

Tracing (1) is from a record on smoked paper.

Tracings (2), (3), (4) and (5) are from the Differential Recording Osmometer.

Tracings (6), (7), (8), (9), (10), (11) and (12) are from the Double Recording Osmometer.

In all tracings except (1) the time tracing marks hours.

In the tracings from the differential instrument, 1 mm. deflection above the zero line, i.e. towards the time tracing, denotes a difference of ·91 cubic millim. between the volumes of fluid passing through the membranes on the pair of discharging osmometers, in favour of the right osmometer; the same deflection below zero or away from the time tracing indicates an excess of ·91 cub. mm. in favour of the left osmometer. In tracings (2), (3) and (4), 95 sq. mm. of skin were exposed on each osmometer; in tracing (5) 190 sq. mm. were used. Irregularities in the line of the tracing are the result of viscosity of the oil used.

In the tracings from the Double Recording Osmometer, $\frac{1}{3}$ mm. rise denotes the introduction of 9 cubic millims. of fluid into the osmometers through the skin. The ebonite supports described in the text were employed in all cases. The reproductions are $\frac{1}{3}$ the size of the actual tracings.

Tracing 1. Resistance to diffusion of curare offered by the fresh gastric mucosa of the frog. The upper row of tracings are taken from the muscle suspended in the tube closed by *fresh*, the lower from that closed by 16 hrs. *stale* mucous membrane. The nerves of both muscles were stimulated by the same induction shock. The numbers below the lower line of tracings indicate the distances in centimetres of the primary from the secondary coil of the induction machine. The muscle in the lower tube closed by *stale* mucosa becomes completely curarised at a time when that in the upper tube closed by *fresh* membrane is little affected. Duration of experiment $4\frac{1}{2}$ hrs., Temp. 15° C.

Tracing 2. Contrast of the diffusion of water and normal saline through fresh frog skin in the direction from *without inwards.* Both osmometers were closed by fresh skin having its inner surface towards the glucose solution, which in the case of the right osmometer was made with normal saline, while that in the left was made with water. (*a*) is a record from immediately after removal of the skin till 4 hrs. post mortem. (*b*) is from 6 hrs. post mortem till 11·75 hrs., (*c*) is from 11·75 till 20 hrs., and (*d*) from 20 hrs. till 27·5 after death of the frog. Temp. 16°—19° C. It will be seen that till about 17 or 18 hrs. post mortem more fluid passes into the osmometer filled with the solution of glucose in normal saline than into that filled with water glucose; after this period the reverse obtains. The experiments in Table 5 were conducted at 8·5—13° C. and by 24 hrs., the change which here occurs 17 to 18 hrs. after death had not taken place.

Tracing 3. Contrast of the diffusion of water and normal saline through fresh frog skin in the direction from *within outwards* (reverse of (2)). The fluids in the two osmometers were disposed as in (2); the outer surface of the skin was however towards the glucose solution on each side, (*a*) is a record from 1 hr. after removal of the skin till 7 hrs. post mortem, (*b*) is from 7 hrs. till 13 hrs. post mortem, (*c*) is from 16 hrs. till 22·5 hrs., and (*d*) from 24 till 31 hrs. after death. Temp. variation 18°—21° C. It will be seen that more water than normal saline passes through the skin throughout the experiment.

Tracing 4. Contrast between diffusion of normal saline from without inwards and from within outwards at various periods after death. In all the records deflection towards the time tracing indicates that more fluid has passed from without inwards than in the reverse direction; deflection away from the time tracing indicates that more fluid has passed from within outwards than from without inwards. On the right osmometer the inner

surface, on the left, the outer surface of the skin, was towards the glucose solution. (*b*), (*c*), (*d*) and (*e*) are taken with parts of the skin of the same frog. The area of skin was 95 sq. mm.

(*a*)	Fresh			till	12 hrs.	post mortem.	Temp.	11°—13° C.
(*b*)	24 hrs.	post mortem	till	37	,,	,,	,,	8·5—10° C.
(*c*)	40·5 ,,	,,	,,	53·5	,,	,,	,,	9°—11° C.
(*d*)	58 ,,	,,	,,	70	,,	,,	,,	10°—11° C.
(*e*)	72 ,,	,,	,,	84	,,	,,	,,	10°—12° C.
(*f*)	84 ,,	,,	,,	96·5	,,	,,	,,	11°—12·5° C.
(*g*)	113 ,,	,,	,,	125	,,	,,	,,	12°—13° C.

Up till about 75 to 76 hrs. post mortem (Tracing 4*e*) the skin is seen to be more permeable from without inwards than in the reverse direction (the "first period" in the text); after a short period during which there is no difference in the permeabilities in the two directions, the third period ensues during which permeability is greater from within outwards than from without inwards.

Tracing 5. End of the first, the second, and commencement of the third of the post mortem periods in a rapidly dying skin. The pieces of skin arranged as in (4). 190 sq. mm. employed instead of 95. The skin had been kept 24 hrs. in 5% glucose in normal saline instead of in normal saline as in Tracing 4. Temp. 10°—11° C. In this case the second period occurs as early as 31 hrs. post mortem and is of short duration.

Tracings 6 *and* 7. Action of chloroform on fresh skin. In both cases the drug was added to the fluids diffusing in the lower osmometer. In (6) the *inner* surface of the skin is towards the glucose solution; in (7) the *outer* surface is so placed. The upper or control tube gives in both cases a tracing with a portion of the same skin, but no chloroform was added to the fluids. Temp. variation in (6), 16°—19° C., in (7), 17°—20·5° C. It is to be noted that while in (6) where *inner* surface is towards glucose, and normal saline passing from *without inwards*, chloroform *lessens* the osmotic transfer, in (7) where the *outer* surface is towards glucose and normal saline passing from *within outwards*, the transfer of fluid is *increased* by the addition of the drug.

Tracings 8 *and* 9. Action of alcohol ·1% on fresh skin. The drug as in (6) and (7) was added to the fluids diffusing through the skin on the lower tube. In (8), in which the *inner* surface is towards the glucose solution, addition of alcohol *quickens*, while in (9) where the *outer* surface is so placed alcohol *retards* the osmotic transfer of the normal saline. Temp. variation (8), 19°—22° C., (9), 16°—19·5° C.

Tracing 10. Action of digitaline ·1% on transfer of normal saline from without inwards. The digitaline was added to the fluids diffusing in the lower osmometer. It is to be noted that while slowing is caused for the first 3 or 4 hrs., a quickening of the stream follows, which is to be accounted for

by the rapid destruction of the vitality of the tissues. Temp. variation 22°—27° C.

Tracing 11. *Upper tube*, diffusion of normal saline from *without inwards*. *Lower tube*, diffusion of normal saline from *within outwards*.

Portions from corresponding parts of the fresh skin. Temp. variation 16°—18·5° C.

Tracing 12. Rapid death of the skin of a feeble frog. Inner surface of skin towards the glucose solution in normal saline. At first a rapid flow of fluid occurs into the osmometer, which would appear to be the result of the absorptive force, this is followed by a slowing, which again is succeeded by augmentation of the stream due to the increase of permeability which occurs upon the death of the tissues. Temp. variation 20°—23° C.


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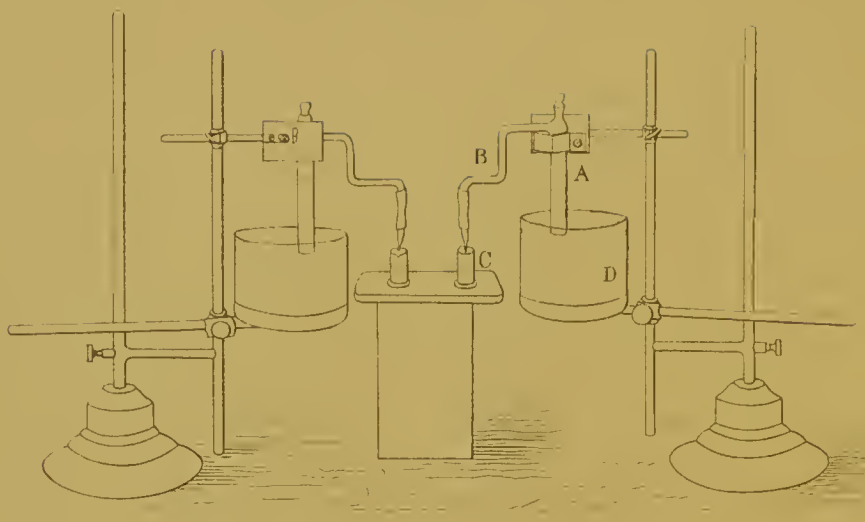

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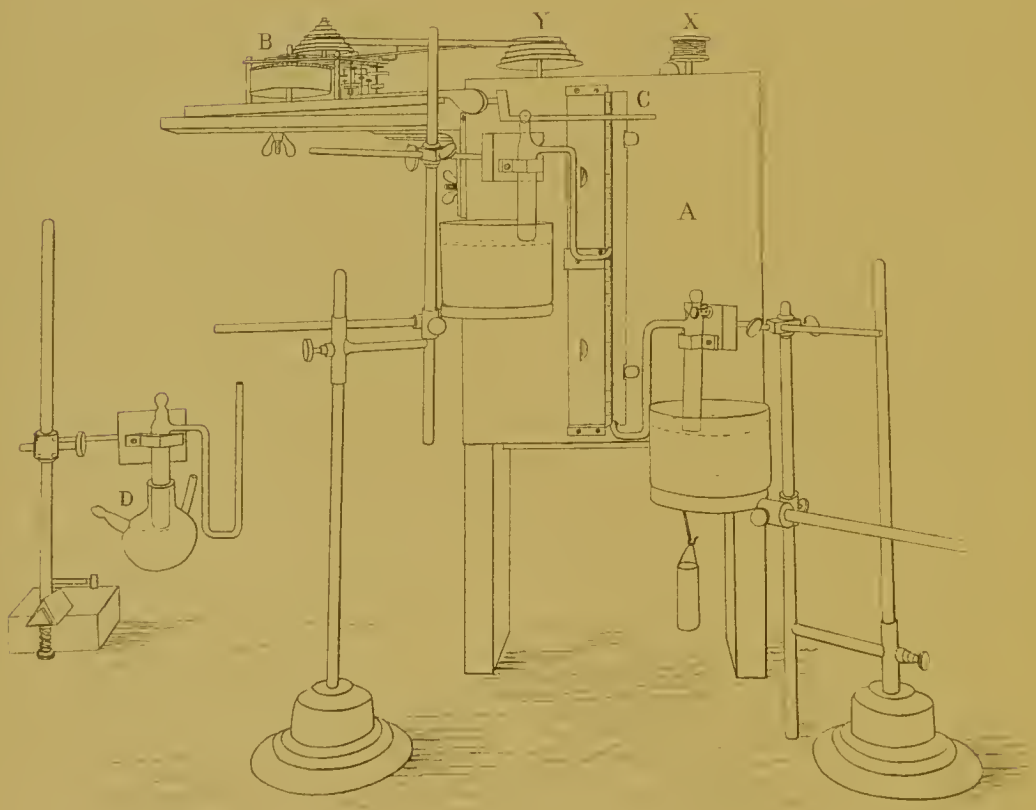

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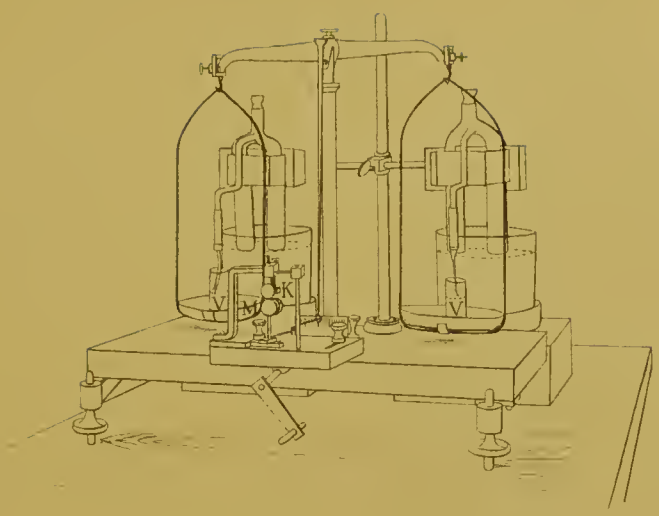

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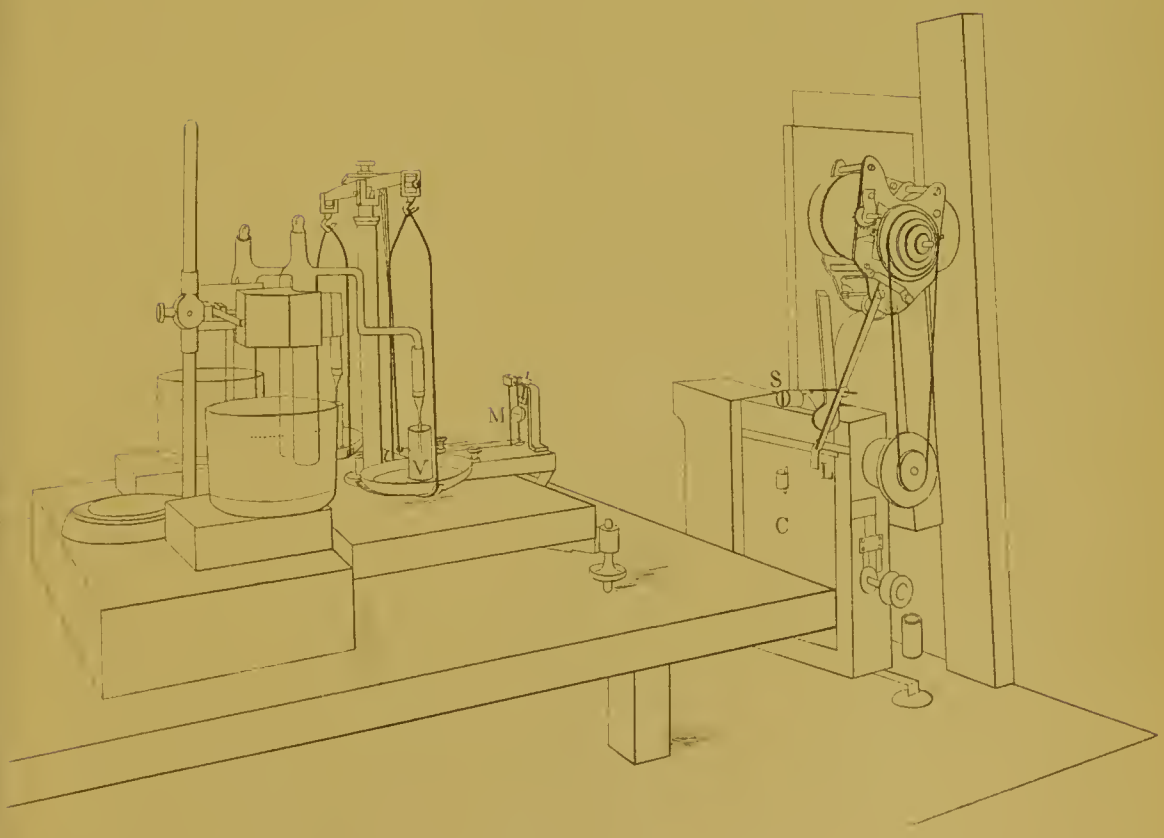

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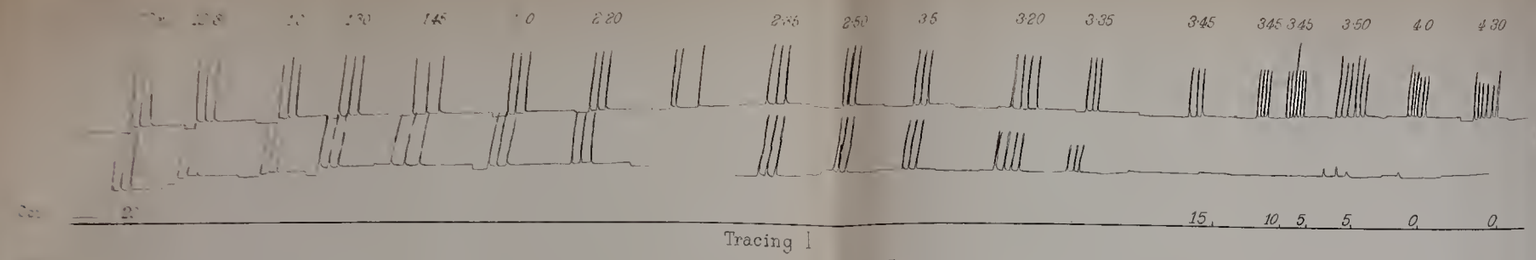

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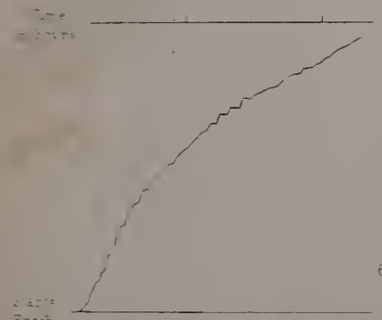

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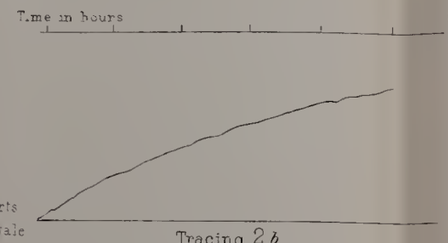

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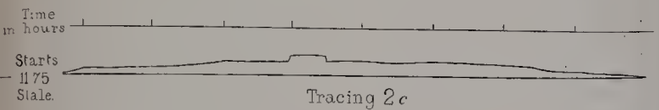

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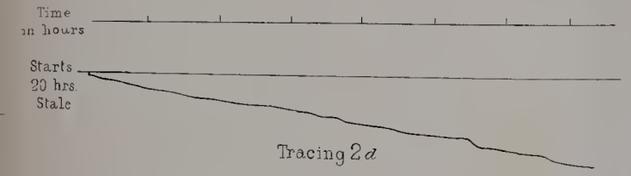

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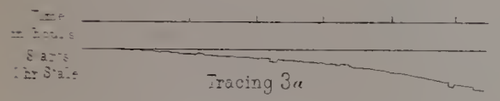

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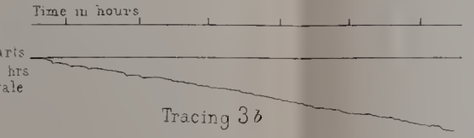

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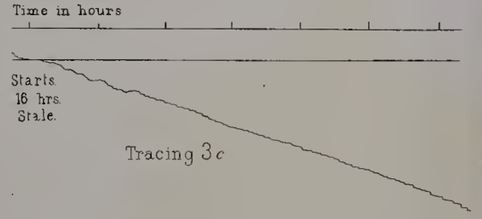

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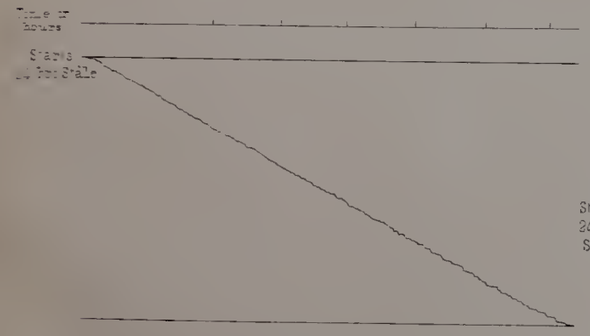

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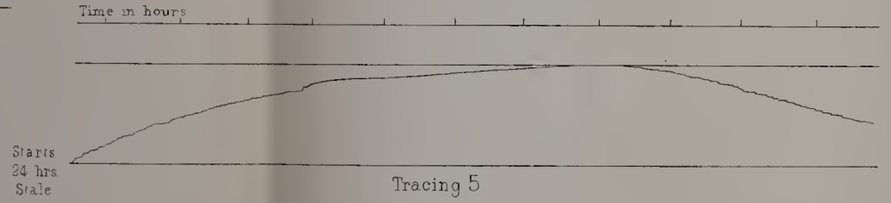

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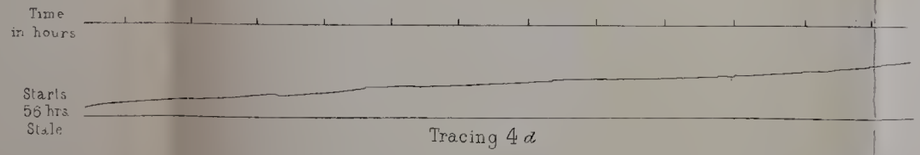

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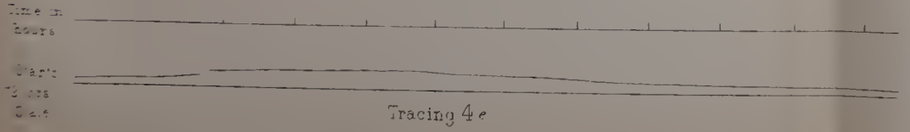

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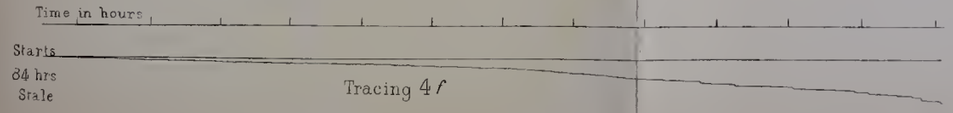

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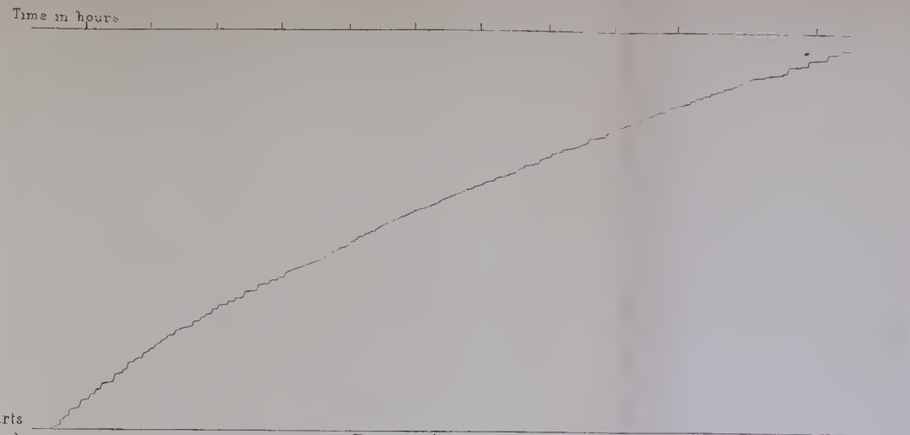

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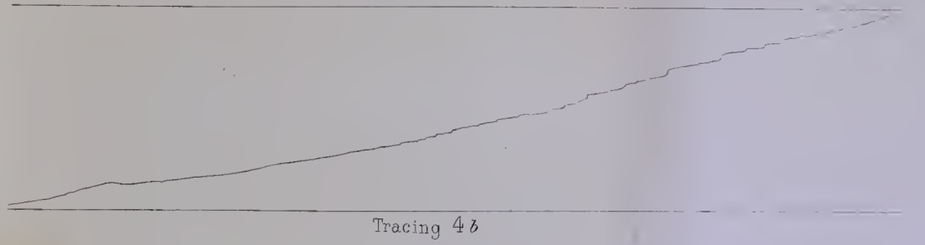

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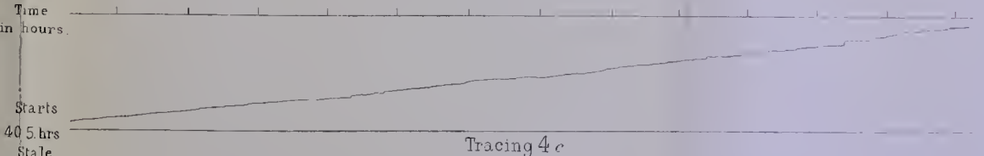

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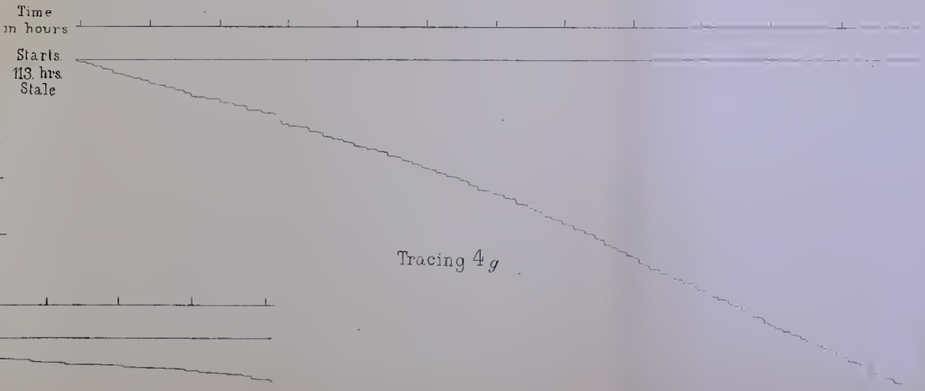

Tracing 4 *g*


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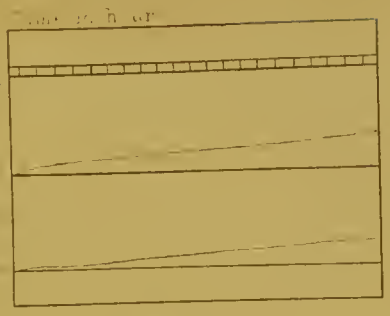

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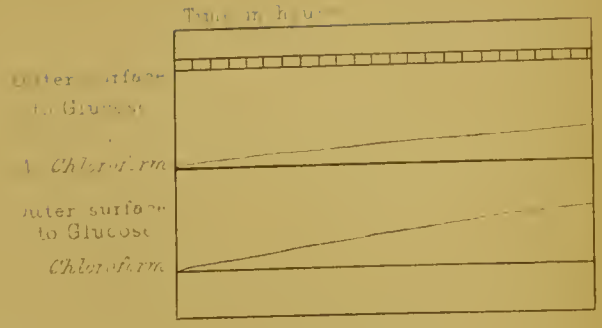

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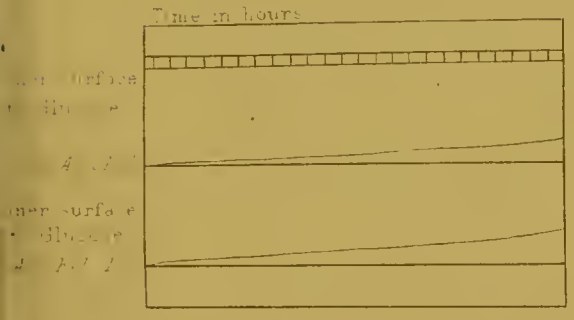

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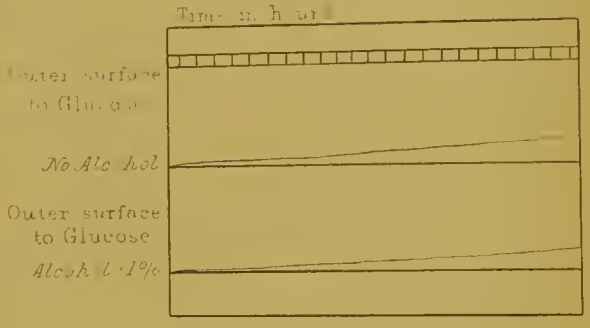

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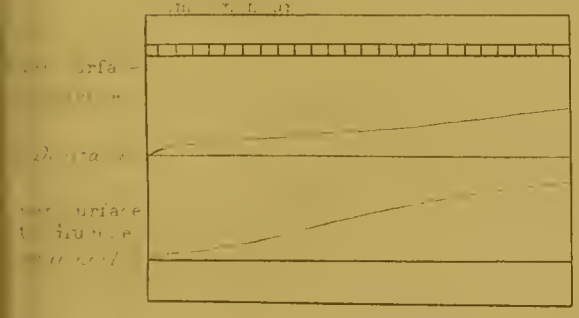

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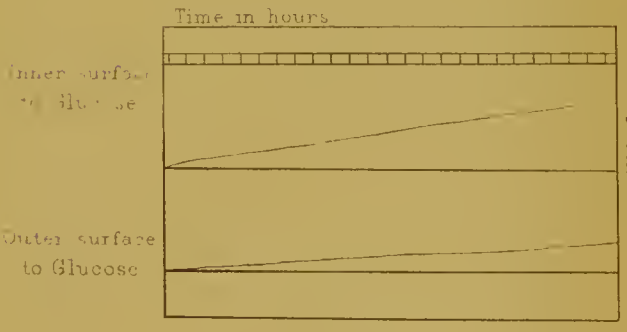

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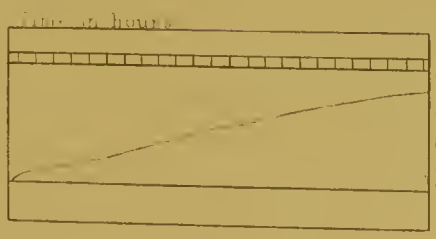

Tracing 12.

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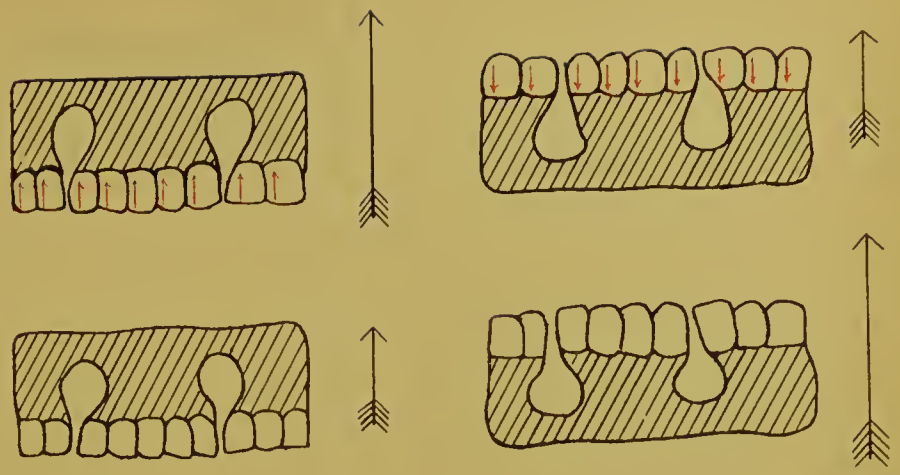
Living Skin

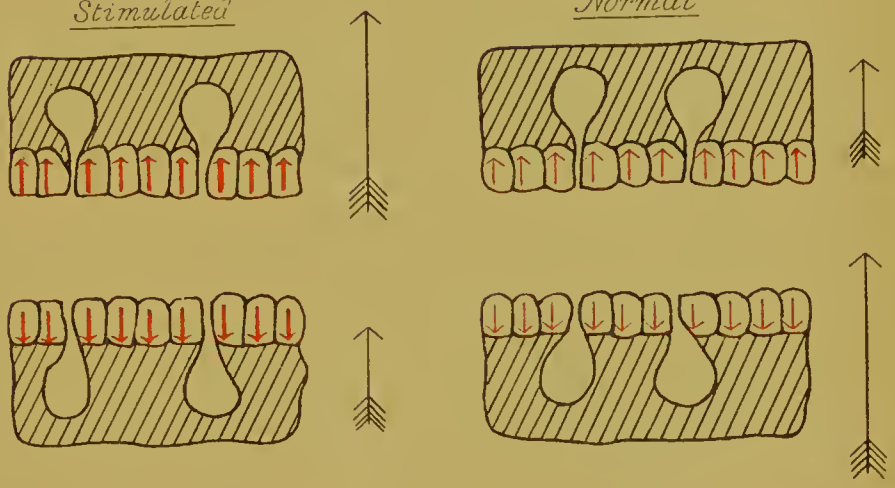
Dead Skin.



Action of a Stimulant on Living Skin.

Stimulated

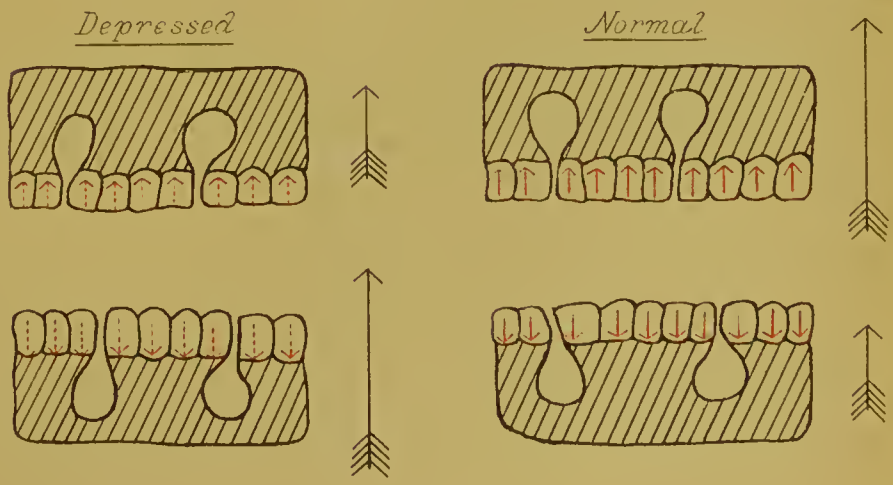
Normal



Action of a Depressant on Living Skin

Depressed

Normal



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